Update of the Provisional Assessment of Perfluorinated Compounds (PFCs) in Drinking Water

Detailed Rationale of the Proposed Levels

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1 Perfluorobutanoic acid, PFBA (375-22-4)

PFBA absorption in rats was estimated at > 95 % after an oral dose of 30 mg/kg. 96 hours after oral administration of 10, 30, or 100 mg/kg ammonium perfluorobutyrate (APFB), male mice eliminated around 35 % in urine and 4–11 % in faeces, and female mice eliminated 65–69 % in urine and 5-7 % in faeces (Chang *et al.*, 2008).

PFBA clearance was examined in workers in the fluorochemical industry who were exposed to various PFBA precursors. For three male workers an elimination half-life ($t_{½}$) of 68 h (around 2.9 days, 95 % CI 41–96 h) was determined as the arithmetic mean. (In the abstract by Chang *et al.*, 2008, elimination half-lives are mentioned for humans of 72.16 h for men and 87.00 h for women; combined 74.63 h. These figures cannot be located in the text.) From the data mentioned by Chang *et al.* (2008) from a slightly larger workplace study with seven men and two women, the Agency for Toxic Substances and Disease Registry (ATSDR, 2009) calculated a mean $t_{½}$ of 72 ± 38 h (for the two women it was 56 and 118 h) and for all 12 volunteers mentioned by Chang *et al.* (2008) a mean $t_{½}$ of 75 ± 38 h. (In ATSDR, 2009, these human data from Chang *et al.*, 2008, are mistakenly given in days in section 3.4.4.2 of the text; the data are correctly presented in hours in Tables 3–8.)

Studies with macaque monkeys (*Macacus cynomolgus*; n = 3 per gender) presented plasma clearance of over 40.32 ± 2.36 h for the males and of 41.04 ± 4.71 h for the females after an intravenous (i.v.) potassium perfluorobutyrate (KPFB) dose of 10 mg/kg.

After administration of an APFB dose of 30 mg/kg by oral gavage to rats, clearance was 1.76 ± 0.26 h for females which means it was five times faster than for the males (9.22 ± 0.75 h).

In mice there was a gender difference in clearance of an APFB dose of 10 mg/kg with factor 4.6 (males: 13.34 ± 4.55 h, females: 2.87 ± 0.30), at 30 mg/kg with factor 5.3 (males: 16.25 ± 7.19 h, females: 3.08 ± 0.26) and at 100 mg/kg with factor 1.9 (males: 5.22 ± 2.27 h, females: 2.79 ± 0.30).

APFB (30 mg/kg) administered by oral gavage to rats presented a roughly 45 % (males) up to 70 % (females) longer clearance period than intravenously (i.v.) administered APFB (males oral: 9.22 ± 0.75 h, i.v.: 6.38 ± 0.53; females oral: 1.76 ± 0.26 h, i.v.: 1.03 ± 0.03 h; Chang *et al.*, 2008).

The data on the rodents are based in each case on only three animals for each dose group.

The monkey/rodent ratio for elimination half-live was around 14 for the female mice (monkey i.v.: 41.04 h vs. mouse oral: 2.87 h) and 40 for the female rats (again i.v.: 1.03 h) or around 6 for the male rats (monkey i.v.: 40.32 h vs. rats i.v.: 6.38 h). On the basis of body weight, ratios of around 4.5 for female mice and 2.5 for female rats were to be assumed (ATSDR, 2009).

PFBA administration of 0.02 % mass fractions in feed (according to ATSDR, 2009, 78 mg/kg·d) to male C57BL/6N mice over 10 days led to an increase in absolute liver weight by, on average, 63 % (from 1.1 ± 0.2 g in the control to 1.8 ± 0.4 g). Body weight increased during this period insignificantly from 23.5 ± 0.7 g to 25.0 ± 0.7 g. This increase in liver

weight was accompanied by changes in the concentrations of the enzymes involved in the drug metabolism (epoxide hydrolase) and/or in the deactivation of reactive oxygen species (ROS) (superoxide dismutase) (Permadi *et al.*, 1992).

Ammonium perfluorobutyrate (APFB) was administered by oral gavage to ten Sprague-Dawley rats in each gender and dose group at doses of 6, 30 or 150 mg/kg·d as formulation MTDID-8391 over 28 days or at doses of 1, 2, 6 or 30 mg/kg·d MTDID-8391 over 90 days. The formulation consisted of an aqueous solution of ammonium perfluorobutyrate with a nominal level of 28.9 % APFB, measured 25.3 % (85–90 % of the target concentration) in the 28-d study and 33.2 % (for the mean dose 6 mg/kg·d, 115 % of the target concentration) in the 90-d study (van Otterdijk, 2007a). For both studies the authors converted this to the PFBA level (van Otterdijk, 2007b). A recovery period of three weeks after the end of dosage was envisaged both in the test of the subacute (for all dose groups) and the subchronic dose (for the high dose group). The liver of the males proved to be the primary target organ with a dose-dependent increase in their weight and microscopic changes (hepatocellular hypertrophy). These effects diminished during the recovery phase. At 150 mg/kg·d APFB over 28 days and at 30 mg/kg·d APFB over 90 days there was an increase in absolute liver weight with no effect on body weight (van Otterdijk, 2007a, b; Butenhoff *et al.*, 2012; these publications are based on the same laboratory studies). The NOAEL in these studies was 6 mg/kg·d after both 28 and 90 days (Butenhoff *et al.*, 2012).

Dose-related follicular thyroid hyperplasia (FTH) and hypertrophy, which did not disappear fully during the follow-up, were also observed in the males. There were no effects in the females. The onset of hepatocellular hypertrophy triggered by activation of the peroxisome proliferator-activated receptor α (PPARα) is a well-known mechanism in rodents. In order to prove this mechanism, specific messenger ribonucleic acids (mRNAs) were assayed in the above-described subacute and subchronic rat studies using polymerase chain reaction (RT-qPCR, reverse transcription quantitative real-time polymerase chain reaction). Here, a PFBA dose-dependent significant increasing transcription of mRNA species was observed, which points to the activation of the PPARα, the CAR (constitutive androstane receptor) and of a thyroid receptor and to a dose-dependent reduced expression of the Ah receptor-regulated cytochrome P450 1A1 (Butenhoff *et al.*, 2012).

To investigate the role of PPARα in the toxic effect of PFBA, Foreman *et al.* (2009) used PPARα wild-type mice (+/+), PPARα-deficient mice (-/-) and mice which express human PPARα. For each group ten male mice were administered 35, 175 or 350 mg/kg·d by oral gavage over 28 days (and bromdesoxyuridine via an implanted minipump during the last seven days). Elevated PPARα gene expression, hepatocellular hypertrophy, liver weights and DNA synthesis in the wild-type and in the mice, which expressed human PPARα, were observed. Furthermore, dose-dependent central liver necrosis was found in the wild-type. The authors suspect a species difference in receptor activation.

In COS-1 cells, into which the PPARα plasmids of mice or humans were transfixed, 5-100 µM PFBA activated the luciferase of the plasmids of both the mice and humans compared to the controls in a concentration-dependent manner. Mouse PPARα reacted more sensitively to PFBA than human PPARα (Wolf *et al.*, 2008). Buhrke *et al.* (2013) confirmed the activation of human PPARα by PFBA; the effect of PFBA was less pronounced than that of PFHxA, PFHpA, PFNA and, in particular, PFOA.

When pregnant CD1 mice were given 35, 175 or 350 mg/kg·d APFB by oral gavage from gestation days 1 to 18, then the following were observed: in the two highest dose groups an elevated number of dams with full litter resorption (11.1 % at 175 mg/kg·d and 29.6 % at 350 mg/kg·d, compared with 6.8 % in the controls) and elevated liver weight after one day, but no longer however ten days after birth, slightly delayed opening of the eyes (around 1.5 days, in all dose groups) and delayed entry into puberty in the high-dose group (Das *et al.*, 2008).

In 127 former and 50 current production workers the PFBA serum concentrations were 73.2 % for the former and 68.0 % for the current workers below the level of quantification (LOQ) (0.5 ng/ml). Only 4 % of the serum samples contained PFBA concentrations of more than 2 ng/ml, maximum 6.2 ng/ml in the former and 2.2 ng/ml in the current employees (Chang *et al.*, 2008).

In the steroidogenesis test PFBA, with a human adrenocortical carcinoma cell line (NCI-H295R), did not lead to the formation of 17β-oestradiol or testosterone and did not react either in the reporter gene tests with human oestrogen, androgen or Ah-receptors (similar to the situation with PFPeA, PFHxA and PFHpA, but in contrast to longer-chain perfluorocarboxylic acids; Rosenmai *et al.*, 2014).

Rationale of the DW_{GV}

The lowest NOAEL of 6 mg/kg·d PFBA from the 90-day study for male rats by Butenhoff *et al.* (2012) was taken as the point of departure (PoD) for the rationale of the DW_{GV}. This 6 mg/kg·d PFBA also proved to be the NOAEL for the males in a parallel 28-day study. However, in the 28-day study APFB increased the occurrence of follicular hyperplasia/hypertrophy of the thyroid gland in the group at 30 mg/kg·d only minimally (nine out of ten animals), at 150 mg/kg·d minimally/slightly (in seven out of ten animals), at the same dose in a 90-day study gradually more clearly (slightly in five out of ten animals; van Otterdijk, 2007a, b; Butenhoff *et al.*, 2012). This amplified effect over time applies in a similar manner to hepatocellular hypertrophy (at 30 mg/kg·d after 28 days no occurrence, after 90 days five animals with minimal and four animals with minor hypertrophy). A time extrapolation, therefore, seems to be appropriate. For this the customary factor 10 is used. Furthermore, a factor 8 (different elimination half-lives rats/humans, see also MDH, 2011; Wilhelm *et al.*, 2010) should be included for interspecies extrapolation for toxicokinetic differences, a factor 2.5 for toxicodynamic differences and a factor 10 for intraspecies differences (respectively $10^{0.5}$ or 3.16 for the toxicokinetic and toxicodynamic differences; WHO, 2005) should be included.

For further assessment there are two uncertainties:

1. It is possible to do without factor 2.5 for toxicodynamic differences in the case of an enlarged liver caused by peroxisome proliferation because of the greater sensitivity of rodents than humans. In the study by Butenhoff *et al.* (2012) one case of an additional coagulation necrosis occurred in the recovery group (150 mg/kg·d) which, because there was also one such case in the recovery group of the control, was not deemed to be toxicologically relevant. In the 28-day study (minimal) follicular hyperplasia/hypertrophy of the thyroid gland occurred. The UDP-glucuronosyl transferase 1A1 was shown to be significantly elevated after doses of

30 or 150 mg/kg·d, as was UDP-glucuronosyl transferase 1A at the highest dose. However, UDP-glucuronosyl transferase 1A6 and UDP-glucuronosyl transferase 2B were statistically significantly reduced at 150 mg/kg·d. The rats were shown to be more sensitive as well to the effect of the concentrations on liver enzymes.

2. Gebbink *et al*. (2015) point to drinking water as the main intake route for PFBA (88–99 %). However, the authors also refer to the limited data available on other intake routes like food. Nevertheless, this could be grounds for a higher even if not, by way of precaution, 90 to 100 % quota of a tolerable dose for drinking water than the customary 10 %.

Depending on the assessment of these two points (consideration of factor 2.5 for toxicodynamic differences yes or no; higher quota of drinking water share in the tolerable dose yes or no), the result for a tolerable dose can be between 3 µg/kg·d (with the assumption of toxicodynamic differences, EF_{total} 2,000) or a drinking water concentration of around 10 µg/l (drinking water quota 10 %) and 7.5 µg/kg·d (no assumed toxicodynamic differences, EF_{total} 800) or around 52 µg/l (drinking water quota 20 %).

According to the "Stocktaking: cases with PFC loads in soil and groundwater in North Rhine Westphalia" from October 2015 (NRW, 2015), maximum PFBA concentrations of around 0.3 µg/l were found in soil and groundwater. In the vast majority of cases PFBA concentrations in the one-digit ng/l-range were measured in soil and drinking water (Gellrich, 2014).

Given these concentrations a DW_{GV} at the lower end of the above-mentioned range, i.e. 10 µg/l, is suggested in line with the concept of the precautionary principle in the Drinking Water Ordinance (section 6(3) (*Trinkwasserverordnung* - TrinkwV) and in relation to the assessment results for other PFCs (Table 1).

Quantitative human toxicological assessments by other institutions

The LUBW (2014) derived "PFC test values for the contamination routes soil – human and soil – groundwater". For PFBA it refers to a drinking water guide value of the German Environment Agency of 7 µg/l (UBA, 2009, 2011). This value is based on the assumption of an identical potency to perfluorooctanesulfonate (PFOS) but different elimination rates. It was outlined in detail that PFOS is eliminated 200 times faster, PFBA only 10 times faster by rats than by humans (Dieter, 2007). As the ratio of elimination rates is included in the extrapolation of data from rats to humans, a 20-fold lower extrapolation factor could be used. Hence, a TDI equivalent value of 0.1 µg/kg·d for PFOS corresponds to a TDI equivalent value of 2 µg/kg·d for PFBA. In the event of a 10% allocation of this value to drinking water consumption, 2 litres consumption per day and 70 kg body weight, this results in a value of 7 µg/l.

The MDH (2011) derived standards for PFBA in groundwater. For chronic exposure it determined an insofar acceptable dose of 2.9 µg/kg·d on the basis of a point of departure (PoD) of 6.9 mg/kg·d from a 90-day oral gavage study in rats with conversion to a human equivalent dose of 0.86 mg/kg·d (divisor 8 because of different elimination half-lives with 3 days for humans and 9.22 h for male rats) and inclusion of an extrapolation factor 30 (factor 3 for toxicodynamic interspecies differences, factor 10 for intraspecies variability) and a safety factor 10 (insufficient data). With an allocation

of 20 % and a drinking water intake rate of 0.043 l/kg·d, this results in a health risk limit of 13.49 µg/l. The Minnesota Department of Health finally states the value determined for short-time exposure of 7 µg/l for chronic exposure too.

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2 Perfluoropentanoic acid, PFPeA (2706-90-3)

Bull *et al.* (2014) give an overview of the data available on PFPeA. No information was found on toxicokinetics, toxicology in laboratory animals or on human toxicology.

In COS-1-cells, into which PPARα-plasmids of mice or humans were transfixed, 0.5-100 µM PFPeA activated the luciferase of the plasmids in mice and humans compared to the controls in a concentration-dependent manner. The PPARα of mice reacted to PFPeA only slightly less sensitively than the human PPARα (Wolf *et al.*, 2012).

In the steroidogenesis test, PFBA with a human adrenocortical carcinoma cell line (NCI-H295R) did not lead to the formation of 17β-oestradiol or testosterone and did not react either in the reporter gene tests with human oestrogen, androgen or Ah-receptors (similar to the situation with PFBA, PFHxA and PFHpA, but in contrast to longer-chain perfluorocarbonic acids; Rosenmai *et al.*, 2014).

No suitable human toxicological data are available for the rationale of a DW_{GW}.

Rationale of a health-related indication value (HRIV)

Given the lack of data no DW_{GV} can be derived for PFPeA. Nor are there any data-backed indications for an HRIV either (Grummt *et al.*, 2013; UBA, 2003). Compared to the toxicity of other rather short-chain PFCs, an HRIV of 3.0 µg/l is proposed, also from the precautionary angle, in acceptance of the proposal by the UBA (2011) and Wilhelm *et al.*, (2010).

Quantitative human toxicological assessments by other institutions

The UBA (2011) mentions in conjunction with PFPeA an HRIV of 3.0 µg/l after an interpolation of its guide values for other PFCs.

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3 Perfluorohexanoic acid, PFHxA (307-24-4)

Bull *et al.* (2014) give an overview of the human toxicological data on PFHxA.

According to studies by Chengelis *et al.* (2009a) PFHxA had a mean elimination half-life ($t_{½}$) in macaque monkeys (*Macacus cynomolgus*; n = 3 per gender) of 5.2 h (standard deviation SD 2.5 h) in males and 2.4 h (SD 1.7 h) in females. In rats a $t_{½}$ of 2 h and 1.9 h at 50 mg/kg·d, of 2.1 h and 2.2 h at 150 mg/kg·d and of 2.9 and 3.0 at 300 mg/kg·d was determined for both males and females respectively; the authors indicate a mean half-life of 2.35 h across all doses and for both genders (Chengelis *et al.*, 2009a). Russell *et al.* (2013) give 1-2 h as the $t_{½}$ for PFHxA for mice and rats and 1-2 d for macaque monkeys. Russell *et al.* (2013) derived the human geometric mean of 32 d from the PFHxA fall-off curves for seven ski wax technicians in one ski season (range 14–49 d). The database for this comes from Nilsson *et al.* (2010a, b, 2013).

The *in vitro* study of the induction of oxidative DNA damage and the potential for the formation of reactive oxygen species (ROS) in human hepatoma cells Hep G2 at up to 2000 µM PFHxA did not present any effects in comparison to the controls (Eriksen *et al.*, 2010). Sodium perfluorohexanoate (Na-PFHx) proved to be non-mutagenic in *in vitro* studies with and without a metabolic activation system with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and with the *Escherichia coli* strain WP2uvrA (333, 667, 1000, 3333 and 5000 µg/plate). In peripheral human lymphocytes (5 to 3860 µg/ml) it did not provoke any chromosome aberrations (Loveless *et al.*, 2009).

In COS-1-cells, into which PPARα plasmids of mice or humans were transfixed, 0.5-100 µM PFHxA activated the luciferase of the plasmids in mice and in humans compared to the controls in a concentration-dependent manner. The PPARα of mice reacted to PFHxA only slightly less sensitively than to human PPARα (Wolf *et al.*, 2008). With PPARα plasmids of mice the concentration dependency of up to 1024 µM was shown; binary mixtures of PFHxA and PFOA had an additive effect at low concentrations (Wolf *et al.*, 2014). Buhrke *et al.* (2013) confirmed the activation of human PPARα by PFHxA; the effect of PFHxA was only exceeded by PFHpA and PFOA.

Kudo *et al.* (2000) examined the potency of the induction of peroxisome-β-oxidation by various PFCs *in vivo*. Of the C6 up to C9 PFCs examined PFHxA showed the least activity, i.e. β-oxidation and liver enhancement increased with chain length (Kudo *et al.*, 2006).

In the steroidogenesis test with a human adrenocortical carcinoma cell line (NCI-H295R) PFHxA did not lead to the formation of 17β-oestradiol or testosterone and did not react either in the reporter gene tests with human oestrogen, androgen or Ah-receptors (similar to the situation with PFBA, PFPeA and PFHpA, but in contrast to longer-chain perfluorocarbonic acids; Rosenmai *et al.*, 2014).

In a 90-day study doses of 10, 50 or 200 mg/kg·d PFHxA were administered by oral gavage to groups of ten Crl:CD(SD) rats (Chengelis *et al.*, 2009b). Reduced body weight gains were registered across all dose groups, but were not attributed to the test substance. Changes were seen with lower parameters for the red blood cells, higher reticulocyte counts and reduced globulin levels at 200 mg/kg·d. In the males in the two upper dose groups there were higher liver enzyme values, in the highest dose group reduced total protein, an elevated albumin/globulin ratio and reduced cholesterol and calcium concentrations in serum. This group also presented minimal centrilobular hypertrophy of hepatic cells and, in correlation with higher liver weight and slightly higher peroxisomes, at the end of the dose period slightly (1.37-fold) elevated β-oxidation activity. Based on liver histopathology and liver weight changes, the authors envisaged a NOAEL of 50 mg/kg·d for males and 200 mg/kg·d for females in this study.

In a 90-day reproduction study Sprague-Dawley rats were given daily doses of 20, 100, or 500 mg/kg Na-PFHx by oral gavage. The control and the high-dose groups contained 55 animals, the two low-dose groups 45 animals each per gender. Sub-groups of five animals for each dose and gender (including the control group) were then observed over a one- and up to three-month recovery period. From 100 mg/kg·d nasal lesions were observed, resulting in an NOAEL of 20 mg/kg·d. No effects were observed with regard to neurological behavioural parameters. Na-PFHx also induced moderate hepatic peroxisomal β-oxidation whereby for this an NOEL was given of 20 mg/kg·d for male rats and of 100 mg/kg·d for female rats. In the male animals in the 500-mg/kg·d group this was also observed even after a 1-month recovery phase (Loveless *et al.*, 2009).

For the reproduction study by Loveless *et al.* (2009) with Na-PFHx, rats were given doses from around 70 days prior to cohabitation until after gestation and lactation for a total of approximately 4 months. No effects on reprotoxic parameters were observed. For the parental generation this resulted in an NOAEL of 20 mg/kg·d because of reduced body weight at higher doses. The NOAEL for reprotoxicity, which was restricted to reduced body weight in the first filial generation, was 100 mg/kg·d. In the developmental toxicity study dosage was administered during gestation days 6-20 (Loveless *et al.*, 2009).

Because of the body weight effects at 500 mg/kg·d, the dam and foetal NOAELs were 100 mg/kg·d.

A chronic oral gavage study over 104 weeks was conducted with PFHxA on toxicological and carcinogenic effects (Klaunig *et al.*, 2015). For this, male Sprague-Dawley rats were given daily doses of 2.5, 15 or 100 mg/kg·d and female rats 5, 30 and 200 mg/kg·d PFHxA. Each dose group, including the control groups, contained 60 animals. The groups with the respective highest dosages contained 70 animals. No effects were observed on body weight, feed intake, functional parameters (including various hormones) or motor activity. The survival rate for the males did not differ from that of the control group; in the females a dose-dependent decrease was observed; it was attributed rather to mechanical or reflux injuries caused by or after oral gavage than seen as an effect of PFHxA. Changes in urine parameters in the two high-dose groups were probably linked to the substance according to the authors, possibly caused by the reduced ability to concentrate urine (females, 200 mg/kg·d) or as a consequence of the low urine pH (males, 100 mg/kg·d). PFHxA-dependent changes in kidney histology were registered in the 200-mg/kg·d groups of females (mainly papillary necrosis, mild form of tubule degeneration). In this group minor haematological changes were also observed (reduced erythrocytes and elevated reticulocytes) (examinations after 25, 51 or 104 weeks). Most of the milder effects did not demonstrate any dose or time correlation. There were no signs of a treatment-related tumorigenic effect of PFHxA.

With regard to the histological kidney effects in females the dose of 30 mg/kg·d from the study by Klaunig *et al.* (2015) is given as the NOAEL. In two notifications of polyfluorinated polymers, within the framework of chemicals notification in Australia, mention is made, *inter alia*, in conjunction with PFHxA of a 2-year toxicity/carcinogenicity study from 2010, possibly the one published by Klaunig *et al.* in 2015. Mention is made there of an NOAEL of 15 mg/kg·d for male rats and of 30 mg/kg·d for female rats (NICNAS, 2014, 2015). A publication by the ENVIRON International Corporation (EIC, 2014) also reports on a 24-month study with the same dose as with Klaunig *et al.* (2015). It likewise mentions the fact that the authors give an NOAEL of 15 mg/kg·d for the males and 30 mg/kg·d for the females. In addition, the NOAEL is linked in the case of females to the histological kidney effects at 200 mg/kg·d and in the case of males to the lower urine pH values at 100 mg/kg·d. According to the data by Klaunig *et al.* (2015) the urine pH values fell in the 52nd study week as the dose was increased and achieved statistical significance at 100 mg/kg·d (control: pH 6.8 ± 0.35, 100 mg/kg·d; pH 6.5 ± 0.39). The values from the 26th week do not fall clearly with the increasing dose, but do also achieve statistical significance at the highest dose, whereby this was more pronounced than after 52 weeks (26 weeks: $p < 0.01$; 52 weeks: $p < 0.05$). Klaunig *et al.* (2015) do not give an NOAEL.

The reprotoxicity of ammonium perfluorohexanoate (APFHx) in mice was investigated by Iwai and Hoberman (2014) in two dose regimens. Doses of 100, 350 or 500 mg/kg·d were administered by oral gavage in Phase I to 20 females at each dose and in Phase II also to 20 females at each of the doses of 7, 35 or 175 mg/kg·d, always from the 6th to the 18th day of gestation. 350 and 500 mg/kg·d led to mortality, elevated salivation and, compared to the controls, changes in body weight gain. At all doses offspring body weights were reduced, but in a permanent manner only at the two highest doses. In addition, at 350 and 500 mg/kg·d stillbirths, reduced viability indices and delayed

development were observed. 175 mg/kg·d caused stillbirths, mortality and reduced body weight in offspring. The NOAEL with reference to maternal and reprotoxicity was 100 mg/kg·d ammonium perfluorohexanoate (95 mg/kg·d PFHxA).

Rationale of the DW_{GV}

Based on the NOAEL of 15 mg/kg·d proposed by NICNAS (2014, 2015) and EIC (2014), probably from the 2-year study (male rats), a human equivalent dose of 1.84 µg/kg·d can be determined with interspecies extrapolation (for toxicokinetics in line with the elimination half-lives human/rat: 768 h (32 d) / 2.35 h ≈ factor 327, and for the toxicodynamics factor 2.5) and with intraspecies extrapolation (respectively $10^{0.5}$ or 3.16 for the toxicokinetic and toxicodynamic differences; WHO, 2005). This results in a DW_{GV} of 6.42 or rounded 6 µg/l with the customary main parameters (70 kg body weight, 2 litres drinking water consumption per day, 10 % allocation of the tolerable human intake only for drinking water).

Russell *et al.* (2013) note that, because of the direct proportionality of the rate of PFHxA excretion with the elimination half-life ($t_{½}$) determined by them and the far more favourable PFHxA clearance rate, it might be advisable to reduce the default extrapolation factor four for the interpecies differences in kinetics. This can only be understood in relation to the other PFCs ("far more favourable" than?), and only when the default factor four was used there. In the case of a mean $t_{½}$ of 2.35 h obtained across all doses and for both genders for the rat as the starting species (Chengelis *et al.*, 2009a) and a mean $t_{½}$ of 32 days for humans as the "target species" (Russell *et al.*, 2013), this results in a species difference of around 327. This factor is, therefore, to be used in the derivation.

Quantitative human toxicological assessments by other institution

There are no known assessments by other institutions.

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4 Perfluoroheptanoic acid, PFHpA (375-85-9)

ATSDR (2009) and Bull *et al.* (2014) give an overview of the human toxicological data on PFHpA.

The half-life after a single injection of 48.64 mmol/kg body weight measured in Wistar rats was 0.10 ± 0.05 days for males (according to ATSDR, 2009, 2.4 ± 1.2 h) and 0.05 ±

0.01 days for females (according to ATSDR, 2009, 1.2 ± 0.2 h); the difference between the genders was statistically significant (Ohmori *et al.*, 2003).

For humans Russell *et al.* (2015) derived 70 d as the geometric mean (range 31 – 123 d) from the PFHpA fall-off curves for five ski wax technicians after one ski season. The database for this came from Nilsson *et al.* (2010a, 2010b, 2013). For a cohort of 31 men and older women from the population in China, Zhang *et al.* (2013) determined an elimination half-life (arithmetic mean) of 1.2 years (standard deviation 0.2 years). Younger women had a similar elimination half-life (arithmetic mean 1.5 years, standard deviation 0.8 years; n = 12).

In the steroidogenesis test with a human adrenocortical carcinoma cell line (NCI-H295R) PFHxA did not lead to the formation of 17β-oestradiol or testosterone and did not react either in the reporter gene tests with human oestrogen, androgen or Ah-receptors (similar to the situation with PFBA, PFPeA and PFHpA, but in contrast to longer-chain perfluorocarbonic acids; Rosenmai *et al.*, 2014).

In COS-1 cells, into which the PPARα plasmids of mice or humans were transfixed, 0.5-30 µM PFHpA activated the luciferase of the plasmids of both mice and humans compared to the controls in a concentration-dependent manner. Mouse PPARα only reacted slightly more sensitively to PFHpA than human PPARα (Wolf *et al.*, 2012). Buhrke *et al.* (2013) confirmed the activation of human PPARα by PFHpA; the effect of PFHpA was only exceeded here by PFOA.

Kudo *et al.* (2000) examined the strength of induction of peroxisome β-oxidation in close connection with PPARα activity (Post, 2014) by means of various PFCs *in vivo*. Out of the examined C6 up to C9 PFCs, PFHpA showed the third most pronounced effect (i.e. β-oxidation and liver enlargement increased with chain length, Kudo *et al.*, 2006).

There are no relevant data for the rationale of a DW_{GV}.

Rationale of the health-related indication value (HRIV)

Because of the lack of data no DW_{GV} can be derived from the human toxicological angle. There are scarcely any indications either for the determination of an HRIV (Grummt *et al.*, 2013; UBA, 2003). Given the potential effect of other PFCs, e.g. on genotoxicity, and the low elimination half-life in rodents, an HRIV of 0.3 µg/l is proposed.

Quantitative human toxicological assessments by other institutions

The LUBW (2014) engages in the following similar consideration in conjunction with the question of a health-related indication value. Similar to the case of PFBA, one major difference for other perfluoroalkane carboxylates, too, is the elimination rate from the human body which depends on the number of perfluorinated carbon atoms. The LUBW mainly relies on the observation by Lud *et al.* (2010), who believe that the following grading of elimination rates is possible: PFBA ≤ PFPA ≤ PFHxA << PFHpA = PFOA for carboxylic acids with 3 up to 7 perfluorinated C atoms. Furthermore, similar to the procedure for PFBA, a comparable potency is also assumed for PFHpA which is similar to that of PFOA (and PFOS). Based on the same, extremely slow elimination rate, the

PFOA drinking water value is applied to PFHpA. This would result in an identical value of 0.3 µg/l.

The UBA (2011) recommends, after an interpolation of its guide values for assessable PFCs in line with the chain length, an HRIV of 0.3 µg/l for PFHpA (Wilhelm *et al.*, 2010).

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5 Perfluorooctanoic acid, PFOA (335-67-1)

The IARC rates PFOA as possibly carcinogenic to humans (group 2B, IARC, 2016). PFOA induces liver, testicular and pancreatic cancer in animal experiments (ATSDR, 2015; Borg and Håkansson, 2012; ECHA, 2015; EPA OPPT, 2005). In an equally potent manner to PFOS, it increases β-oxidation of fatty acids, catalase activity, omega and omega-minus-1 hydroxylation of lauric acid, cytosolic epoxide hydrolase and DT diaphorase in liver peroxisomes (Sohlenius *et al.*, 1993). This leads to PFOA-mediated peroxisome proliferation. As PFOA cannot, in principle, be broken down by β-oxidation because of its fluorine atom in an α position, this leads to excess peroxisomes and highly reactive oxygen. After a series of further morphological and biochemical changes, the consequences are then liver enlargement and tumours (Dieter, 2007).

The biochemical mechanisms responsible for carcinogenesis (peroxisome proliferation, but also disruption of sex hormone levels) are very probably irrelevant for the assessment of human carcinogens. The types of tumours which presented in animal experiments with PFOA were not observed even in highly exposed human cohorts up to around 2006 (Dieter, 2007). This gap was not closed until the work by the C8 Science Panel (2014), which conducted epidemiological studies to clarify the health relevance of PFOA emissions from 2005 up to 2010 in the Ohio River Valley (West Virginia, USA). Within the framework of these studies the patient data collected in questionnaires and the PFOA blood levels of 69,030 volunteers were evaluated. 74 % of them took part in the follow-up studies in the years 2009–2011. As a consequence of different study designs and the multiple evaluation strategy, the research team came to the conclusion that there was a probable link between the incidence of testicular and kidney cancer and the level of PFOA exposure. Furthermore, the PFOA blood concentration correlated with the occurrence of high cholesterol levels, chronic inflammatory bowel disease, a thyroid disorder and with pre-eclampsia, i.e. a hypertensive pregnancy intoxication. Up to now it has been assumed that carcinogenesis is caused by a mechanism of action for which an effect threshold can be assumed (Dieter, 2007).

In the case of PFOA liver effects, immunological effects and effects on embryonic development are the most sensitive endpoints and rats and mice are the most sensitive species. The known mechanism of peroxisome proliferation by means of activation of the peroxisome proliferator-activated receptor alpha (PPARα) in rodents triggers the liver effects and some of the developmental toxic effects. Numerous molecular points

of attack and mechanisms can suppress the production of immunoglobulin M (IgM) against antigens. However, PPARα is the primary isoform of the receptor in lymphocytes; in particular B cells, and PFOA and PFOS are known PPARα agonists. More recent studies have shown that PPARα agonists (including the inhibitor WY14.643 and PFOA) have major effects on the immune response of mice, in particular they inhibit the production of IL-6[1], TNF-α[2] and interferon-γ[3], and reduce spleen weight, the number of spleen-related white blood cells and the production of antibodies after antigen provocation. PPARα also inhibits the expression of inflammation genes like cyclo-oxygenase-2 and endothelin-1. With the help of PPARα-null mice it was shown that the WY14.643-mediated increase in TNF-α in plasma and the PFOA-induced suppression of Con-A-induced lymphocyte proliferation depend on PPARα. Hence the PPARα definitively plays a role in immune suppression, too (Peden-Adams *et al.*, 2008).

Humans react less sensitively to the PPARα agonists than rats and mice. The derivation of assessment criteria for rodent data could, therefore, lead to more cautious values. Monkeys, which also react less sensitively to PPARα agonists, are to be given priority as a suitable model for humans (ATSDR, 2015). A subchronic 26-week study with PFOA in macaque monkeys (Butenhoff *et al.*, 2002) agrees in terms of symptoms with the results for rats and mice. In the rodent studies, however, developmental and immunotoxicological endpoints were also investigated, but this was not done in the monkey study. As the developmental toxicological endpoints in rodents did not react more sensitively than those for liver, and the pathological relevance of immunotoxicological effects is unclear, an assessment criterion for PFOA based on liver toxicity is very probably protective (ATSDR, 2015).

Foundations for the rationale of a DW_{GV}

Animal studies

Based on the 26-week study with monkeys (Butenhoff *et al.*, 2002):

In the study by Butenhoff *et al.* (2002) male macaque monkeys were given, over a period of 26 weeks, daily doses of 0, 3, 10 or 30 mg/kg·d ammonium perfluorooctanoate (APFO) as capsules and after 5 weeks the PFOA concentration was measured in serum. The only organ weight change observed was a dose-dependent increase in absolute liver weight across all dose groups. The PFOA serum concentrations were linked to the doses and the appropriate human equivalent doses were calculated by means of the different elimination half-lives of monkeys and humans (ATSDR, 2015). With the help of the Benchmark Dose Software (BMDS) of the US-EPA, models were adjusted to the liver weight and serum concentration data and a lower confidence limit (BMDL - benchmark dose level lower bound) of 10 % relative deviation from the benchmark dose was calculated. A $BMDL_{10}$ human equivalent dose of 1.54 µg/kg·d was taken as the point of departure (PoD) for the assessment criterion.

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[1] IL-6 (Interleukin-6): protein which regulates the inflammatory reaction of the body.
[2] TNF-α (tumour necrosis factor-α) is involved in the local and systemic inflammation processes.
[3] Interferon-γ is involved in the inflammation processes and has antiviral, immunostimulating and anti-tumour properties.

An assessment criterion of 25.7 ng/kg·d for lifelong exposure can be derived from this equivalent dose after division by a total factor 60. This total factor results from a factor reduced from 10 for a 90-day study to factor 3 for extrapolation of the 26-week study duration to the entire lifetime, factor 10 for interindividual variability in humans and factor 2 for toxicodynamic species differences with dosimetric adjustment. Here factor 2 was selected, because monkeys are thought to react in a relatively similar manner to humans and because the HED of 1.54 µg/kg·d is already the lowest value from a range of possible equivalent doses which range up to 4.68 µg/kg·d. [The ATSDR (2015) also calculates, in addition, a safety factor 3 which is not customary in Germany for uncertainties in the database because of a lack of developmental toxicological and immunotoxicological data for monkeys.] With an allocation of 10 % for uptake from drinking water, drinking water consumption of 2 litres per day and body weight of 70 kg, the hepatomegaly observed in monkeys results in a lifelong tolerable guide value based on human toxicology of 89.8 or rounded 90 ng/l drinking water (0.0257 µg/kg·d · 0.1 · 70 kg / 2 l/d = 0.08995 µg/l).

Based on the study on developmental toxicity in CD-1 mice (Macon *et al.*, 2011):

Macon *et al.* (2011) examined the developmental toxicological effects of low PFOA doses. To this end, pregnant CD-1 mice were either administered 0, 0.3, 1 or 3 mg/kg·d by oral gavage over the full period of gestation or 0, 0.01, 0.1 or 1 mg/kg·d during the 2nd half of gestation. PFOA significantly increased relative liver weight in offspring (in the 1st sub-study at all doses higher than the control, in the 2nd sub-study from 1 mg/kg·d). In both parts of the study the female offspring of all treated dams presented significantly inhibited growth of the mammary gland epithelium. Analyses of PFOA levels showed elevated concentrations in the serum and liver of the offspring up to 6 weeks after birth. By contrast, the levels were low in the brain and could no longer be detected after 4 weeks. These data show that in CD-1 mice the PFOA effects on mammary gland development started at lower concentrations than the effects on the liver. When the substance was administered over the entire period of gestation they continued for 12 weeks after birth. Because of the sensitive reaction of mammary gland development, no NOAEL could be determined. The LOAEL was 0.01 mg/kg·d. The serum concentration in female offspring in the 2nd sub-study fell continuously; after 21 days it was 16.5 ng/ml which is still significantly higher than the control of 4.1 ng/ml (probably due to ubiquitous exposure). Because of this small difference factor 5 is deemed to be appropriate for extrapolation to an NOAEL of 2 µg/kg·d. An EF for the duration of the experiment is not appropriate, as the time window is the decisive factor for the developmental toxic effect and not the duration of exposure. The HBM Commission takes into account the toxicokinetic difference between mice and humans with factor 7 that was probably determined allometrically (HBM, 2015a).

Yang *et al.* (2009) observed in Balb/C and C57B1/6 mice PFOA effects on mammary gland development at a dose of $\geq$ 5 mg/kg·d but not at $\leq$ 1mg/kg·d.

According to Macon *et al.* (2011) the development of the mammary gland (LOAEL = 0.01 mg/kg·d) reacted more sensitively to PFOA than the liver (LOAEL = 0.3 mg/kg·d) in CD-1 mice. This elevated sensitivity could, however, also be due to the different time window of dosage. One point of attack of PFOA is the PPARα receptor, to which liver toxicity and the general developmental toxic effects of PFOA can probably be attributed (Abbott *et al.*, 2007; Rosen *et al.*, 2009; Wolf *et al.*, 2008). This receptor reacts far more sensitively

to PFOA in rodents than in humans. Zhao *et al.* (2010) showed normal lactation after exposure to PFOA in PPARα knock-out mice. This indicates that the development of the mammary gland could also be regulated via PPARα and, consequently, could react less sensitively in humans than in CD-1 mice. At all events, given the known elevated sensitivity of rodents there is no need for a toxicodynamically justified factor for extrapolation to humans. Inter-individual variability in humans is taken into account with factor 10. This leads to a TDI equivalent value of 28.57 ng/kg·d. With an allocation of 10 % for intake from drinking water, drinking water consumption of 2 litres per day and body weight of 70 kg, this results in a lifelong tolerable guide value based on human toxicology of 99.995 ng/l drinking water (≙ 0.1 µg/l).

The use of data on the development of the mammary gland in mice to justify values is criticised because various mice strains react differently (see Table 5.1 from Chang, 2016). The eight studies available up to now report stimulation, inhibition or no change in mammary gland development under the influence of PFOA. In one study counter-effects were even observed at various doses (Yang *et al.*, 2009).

Table 5.1: Studies on the development of the mammary gland in mice under the influence of PFOA (Chang, 2016)

Study	**Mouse Strain**	***Mammary Gland Development Outcome (per Study Authors)***
White *et al.* 2007	CD-1	*Stunted*
White *et al.* 2009	CD-1	*Delayed*
Yang *et al.* 2009	C57BL6	*Stimulatory (5 mg/kg), Inhibitory (10 mg/kg)*
	Balb/c	*Inhibitory*
Zhao *et al.* 2010	C57BL/6	*Stimulated*
Macon *et al.* 2011	CD-1	*Delayed*
White *et al.* 2011	CD-1	*Delayed*
Albrecht *et al.* 2013	Sv/129 W	*No effect*
	PPARα KO	*No effect*
	hPPARα	*No effect*
Tucker *et al.* 2014	CD-1	*Delayed*
	C57BL/6	*Delayed*

Overall these studies contribute to uncertainty about this endpoint and challenge its relevance. For the current assessment of PFOA it can, therefore, be concluded that the study by Macon *et al.* (2011), if it is at all relevant for humans, constitutes a comparatively sensitive reaction. The value of 0.1 µg/l derived from the toxicological endpoint mammary gland development, therefore, seems to be sufficient.

Compared with the monkey study with a difference between LOAEL (3 mg/kg·d) and TDI (25.7 ng/kg·d) of almost 117,000, there is a far lower difference of 350 from the mice experiment because of a far lower LOAEL of 0.01 mg/kg·d. Therefore, considerable importance is to be attributed to the latter despite the above constraint. Its robustness is increased by the fact that they both come to the same conclusion.

Human epidemiological studies

The Human Biomonitoring Commission (HBM, 2015a) gives a range of 1 up to 10 ng/ml for levels of acceptable intake in blood plasma and then sets an HBM-I value of 2 ng/ml (= µg/l).

If this HBM-I value is understood to be the NOAEL-equivalent concentration for humans [C(t) = 2.0 ng/ml plasma = 2.0 µg/l plasma = 0.002 mg/l plasma], then equation 5 in the PBPK model of the Human Biomonitoring Commission leads to a linking of the daily dose at steady oral intake (TDI) with the steady-state serum concentration.

Equation 5: $C(t) = TDI \cdot \frac{BW}{F} \cdot R(t)$

F = factor D/C_{ss} = 0.00835 L/d for PFOA
(0.0528 L/d for PFOS)
D = dose (TDI equivalent)
C_{SS} = steady-state concentration
$R(t) = 1 - e^{\frac{-(\ln 2)\cdot t}{t_{50}}}$ = 0.527 = time correction
t_{50} = 3.7 years for PFOA (Arnsberg cohort, estimated)
t = 4 years (assumed exposure duration)
BW = body weight (70 kg)

And for the recalculation

Equation 6, with 2 ng/ml: $D \cdot BW = C(t) \cdot \frac{F}{R(t)}$

$$TDI = C(t) \cdot \frac{F}{R(t) \cdot BW}$$

$$TDI_{PFOA} = \frac{0.002 \frac{mg}{l} \cdot 0.00835 \frac{l}{d}}{0.527 \cdot 70\ kg} = 0.000\,000\,453\ mg/kg{\cdot}d \approx 0.5\ ng/kg{\cdot}d$$

Based on these epidemiological data, this results in a concentration of 0.5 ng/kg·d · 0.1 · 70 kg · 1 d / 2 l = 1.57 ng/l ≈ 2 ng/l in drinking water.

A recalculation based on the HBM-I value of 2.0 ng/ml serum is supported by the studies mentioned in Table 5.2 when it comes to lipid metabolism disorders, fertility, immunity and developmental toxicity.

Table 5.2: Effect threshold (PoD) for PFOA derived from human epidemiological studies

References	**Findings**	**PoD** [ng/ml]	**Exposure** [ng/kg·d]	PFOS-PoD [ng/ml]	**Multiple exposure**
Fat metabolism					
Geiger *et al.* (2014a+b)	Increase in total cholesterol, LDL cholesterol (dyslipidaemia); stat. sign. quantile differences; mean=4.2 ng/ml; 815 children	**> 4.7**		> 21.8 $PFOS_{mean}$= 17.7	Double exposure PFOA + PFOS
Nelson *et al.* (2010)	Increase in total cholesterol + nonHDL; $PFOA_{range}$: 0.1 – 37.3 ng/ml; $PFOA_{median}$: 3.9 ng/ml	no PoD		$PFOS_{median}$= 19.9	Multiple exposure PFOA + PFNA + PFHxS + PFOS); PFOA/PFOS: r=0.65
Fitz-Simon *et al.* (2013a+b)	PFOA above DW, PFOS general contamination; reversible total cholesterol↑ for $PFOA_{geoM}$-reduction: 74.8 → 30.8 ng/ml	no PoD		$PFOS_{geoM}$: 18.5 → 8.2	Multiple exposure PFOA + PFOS + PFAS; very important for HBM derivation!
Frisbee *et al.* (2010)	Total cholesterol↑, LDL cholesterol↑. 12476 children PFOA above TW $PFOA_{median}$ = 32.6 ng/ml	**6** (Upper level low quantile with no effect)		**15** Upper level low quantile with no effect $PFOS_{median}$ = 20	Multiple exposure PFOA + PFOS + PFAS;
Steenland *et al.* (2009)	Total cholesterol↑, LDL cholesterol↑; 46294 adults;	**< 13**	3	**< 13**	Double exposure PFOA + PFOS

References	Findings	PoD [ng/ml]	Exposure [ng/kg·d]	PFOS-PoD [ng/ml]	Multiple exposure
	PFAS aboveDW $PFOA_{quartile}$: 13.1/26.5/66.9-17557 $1^{st} - 2^{nd}$ quartiles limit OR=1.21	(HBM, 2015c)		Increase greater than PFOA (HBM, 2015c) $PFOS_{quartile}$: 13.2/19.5/28-760	PFOA/PFOS correlation r = 0.32
Steenland *et al.* (2009); HBM (2015a)	Total cholesterol↑, LDL cholesterol↑; 46294 adults increase for PFOA less pronounced than for PFOS	**30** (HBM, 2015a) $PFOA_{mean}$ = 80	7	$PFOS_{mean}$ = 22	Double exposure PFOA + PFOS
Frisbee *et al.* (2009); Steenland *et al.* (2009)	PFOA production, 60030 persons Hypercholesterinaemia $PFOA_{median}$ = 32.6 ng/ml PFO_{mean} = 70.9 ng/ml	no PoD <142 = 1st quintile		$PFOS_{median}$ = 20	Double exposure PFOA + PFOS
Eriksen *et al.* (2013)	Increase in total cholesterol 753 adults, 50-65 years; $PFOA_{mean}$=7.1 ng/ml $Reference_{95}$ = 10 ng/ml	**3** (stronger than PFOS, HBM, 2015c)		**17** $PFOS_{mean}$=36,1 ($Reference_{95}$ = 20-25)	Double exposure PFOA + PFOS
Starling *et al.* (2014)	Increase in total cholesterol systematic trend for $PFOA_{median}$ = 2.3	no PoD		$PFOS_{median}$ = 13	Double exposure PFOA + PFOS
Fisher *et al.* (2013)	Increase in total cholesterol contrast 1^{st} /4^{th} quartiles	**3.6**		(12.92) no PoD can be derived	Multiple exposure to PFOA + PFHxS + PFOS
Zeng *et al.* (2015)	Increase in total cholesterol	**< 2**		**15 - 20**	Multiple exposure PFOA + PFOS + 6 other PFAS
Developmental toxicity					
Stein *et al.* (2009)	Pre-eclampsia 5262 pregnancies	geoM = 21.2 (<LoQ – 894) n = 1845		geoM =13.6 (<LoQ – 83.4); Pre-eclempsia 1. 3 at >50%ile; 1.6 at > 90%ile) + birth weight↓ (OR = 1.5 at >50%ile; 1.8 at > 90%ile)	Double exposure PFOA + PFOS
Fei *et al.* (2007)	Birth weight↓ $PFOA_{mean}$= 5.6 ng/ml	**3.91**		$PFOS_{mean}$ = 35.3	Double exposure PFOA + PFOS
Washino *et al.* (2009)	Birth weight↓ $PFOA_{geoM}$ = 1.2 ng/ml (<NG – 5.3)	No association		$PFOS_{geoM}$ = 4.9 (1.3 – 16.2) statis. signif. association	Double exposure PFOA + PFOS
Stein *et al.* (2009)	Birth weight↓ $PFOA_{geoM}$ = 21.2 ng/ml (<NG – 894)	No association		$PFOS_{geoM}$ = 13.6 (<NG – 83.4); > median: birth weight↓ pre-eclempsia statis. signif.	Double exposure PFOA + PFOS
Nolan *et al.* (2009)	1555 births: no birth weight↓ no gestation duration↑, no premature births PFOA DW_{mean} = 6.78 µg/L (1.7 – 17.7 µg/L)	Blood $PFOA_{median}$ = 400 = 80 x US-av'ge no association			Double exposure probable but not mentioned
Darrow *et al.* (2013)	1630 births: Birth weight, gestation duration, PIH PIH-OR/1n-unit OR_{PFOA} = 1.27 OR_{PFOS} = 1.47	$PFOA_{geoM}$ = 16.2 (0.6 – 460) PIH-OR/1n-unit OR_{PFOA} = 1.27		$PFOS_{geoM}$ = 13.2 (<NG – 93); PIH-OR/1n-unit OR_{PFOS} = 1.47+ birth weight↓	Double exposure PFOA + PFOS
Olsen *et al.* (2009)	Birth weight, PI + other According to animal exper. BMDL adverse effects would only be expected far above the measured concentration in human studies → confounding through individual physiology of the volunteers or different maternal plasma expansion during gestation?				Mixed exposure to several PFAS + other substances?

References	Findings	PoD [ng/ml]	Exposure [ng/kg·d]	PFOS-PoD [ng/ml]	Multiple exposure
Johnson *et al.* (2014)	Obstetric parameters↓ / 1 ng PFOA/ml; 9 studies: -18.9 g birth weight 5 studies: -0.1 cm body length	no PoD			Mixed exposure to several PFAS
Bach *et al.* (2015)	14 studies of Aug 2004 – Dec 2013: birth weight↓	Association not sufficiently proved		Association not sufficiently proven	Mixed exposure to several PFAS
Verner *et al.* (2015)	Birth weight↓/**ng/ml**↑ PFAS effect overestimated by confounder: glomerular filtration rate	-7.1 instead of -14.7 g		-2 instead of -5 g	Double exposure PFOA + PFOS
Fertility (of special importance!)					
Vélez *et al.* (2014)	TTP $PFOA_{median}$ = 1.7 ng/ml; $PFOA_{max}$ = 16 ng/ml; PFOA 3x, PFOS 14x lower than before	**PoD < 5** PFOA x 1.8 → +30 % TTP		no PoD $PFOS_{median}$ = 4,7 $PFOS_{max}$ = 36	Double exposure PFOA + PFOS
Fei *et al.* (2009)	Delayed fertility (TTP) $PFOA_{median}$ = 5.3 ng/ml $Reference_{95}$ = 10 ng/ml Danish birth cohort, 1240 women	**PoD = 4 – 5** Reference quartile upper level		**PoD = 26** reference quartile upper level $PFOS_{median}$=33.7 $Reference_{women}$ =20	Double exposure PFOA + PFOS → GFS = 100 ng/L
Whitworth *et al.* (2012)	Delayed fertility (TTP) Q_{25}/median/Q_{75} 1.66/2.25/3.03	**2.5**		**17** Q_{25}/median/Q_{75} 10.3/13.1/16,6	Double exposure PFOA + PFOS
Vagi *et al.* (2014)	Polycystic ovarian syndrome (PCOS) PCOS-$PFOA_{mean}$= 4.1 without PCOS-$PFOA_{mean}$=2.3	3rd tercile = **4.1** - 13.4 PCOS-OR 3rd/1st tercile = 6.9		3rd tercile = **8.6** – 27.9 PCOS-$PFOS_{mean}$= 8.2 Without PCOS-$PFOS_{mean}$=4.9 OR 3rd /1st tercile = 5.8	Multiple exposure PFOA + PFOS + PBDE + PCB + OCP + phthalates + BPA
Lyngsø *et al.* (2014)	PFOA impact on cycle length PFOS impact on cycle length variability percentile: 10/50/90 Limited transferability	**2** Poland 1.5/**2.7**/4.3 Greenland 1/**1.8**/3 Ukraine 0.5/**1**/1.7		No PoD Greenland 12/**20.2**/36.6 Poland 5.2/**8**/12.1 Ukraine 2.9/**5**/8	Double exposure PFOA + PFOS
Immunity					
Fei *et al.* (2010)	General infection resistance, no exposure-dependent trend, not to be used for assessment!	$PFOA_{mean}$= 5.6 (LoQ – 41.5)		$PFOS_{mean}$= 35.3 (6.4 – 106.7)	Double exposure PFOA + PFOS
Grandjean *et al.* (2012, 2013)	Diphtheria and tetanus antibodies↓ in 5-7-year-olds 2 x PFAS body burden → 42% – 59% AK reduction contradicts Fei *et al.* (2010): no trend; contradicts Peters & Gonzalez (2010): no weighted TEQ	Maternal PFOA (25/geoM/75): 2.56/3.2/4.01 child PFOA 3.33/4.1/4.96 PFAS-$BMDL_{05}$: **0.3**; no effect below observed values → **1.5**		Maternal PFOS (25/geoM/75): 23.2/27.3/33.1 Child PFOS 13.5/16.7/21.1 PFAS-$BMDL_{05}$: **1.3**; no effect under observed values values → **7**	Multiple exposure PFOA + PFOS + PFHxS + PFNA + PFDA + PCB + MeHg
Looker *et al.* (2014)	Far lower vaccine protection after influenza vaccination; not informative for HBM-I value derivation	**< 14** ≤13.7/31.5/90/2140		No effect ≤5.8/9.2/14.5/42.3	Double exposure PFOA + PFOS
Dong *et al.* (2013)	Decrease in immunity, asthma (IgE, eosinophil granulocytes, eosinophil cationic protein)	**0.4 - 0.5**		**19.6**	Double exposure PFOA/PFOS: r = 0.64
Humblet *et al.*, 2014	Decrease in immunity, 1877 adolescents, median with/without asthma = 4.3/4 ng/ml	no PoD		Median with/without asthma = 17/16.8 ng/ml (not sign.)	Double exposure PFOA/PFOS: r = 0.68

PIH = pregnancy-induced hypertension - without proteinuria), pre-eclempsia: with proteinuria

OR = Odds ratio, PI (ponderal index) = mass/(length^3) ≙ mass/fictive volume
TTP = time to pregnancy

Grandjean *et al.* (2012) observed reduced vaccine protection through inhibition of antibody formation in the case of diphtheria and tetanus vaccinations in 5-7-year-old children. As a consequence of mixed exposure (PFOA + PFNA + PFDA + PFHxS + PFOS) there was a multiple load and the serum concentration was summed up as an indicator of exposure. On this basis HBM (2015c) then derived a feasible point of departure for the assessment of 0.3 ng/ml for PFOA. What goes against this procedure is the criticism by Zobel *et al.* (2012) and Peters and Gonzalez (2011) that the PFC concentration could not be described by toxicological equivalence factors and that the results did not match the results of a similar study by Fei *et al.* (2010; quoted in HBM, 2015c). In the study by Fei *et al.* (2010) the benchmark response "infectious diseases" was, however, understood to be so comprehensive and heterogeneous that it can scarcely be used as a specific indicator for impairment of the immune system. Nonetheless, it seems questionable that the impact on vaccine protection is to be attributed to just one of the components and that the other PFCs would have no impact on antibody formation. Furthermore, the assessed cohort had co-exposure to PCBs and methyl mercury, whose impact cannot be assessed. Consequently, these data are not used to calculate a DW_{GV}.

None of the epidemiological studies was able to examine the effect of an individual substance as PFCs occur in the environment mostly and in the blood serum of human individuals generally as mixtures of several individual substances. In particular the occurrence of PFOS and PFOA is deemed to be ubiquitous (UBA, 2016). The ATSDR (2015) gives the mean concentrations listed in Table 5 for PFOA, PFOS and PFHxS in blood serum for the general population of the USA. The concentrations of other PFCs (PFBA, PFHpA, PFNA, PFDeA, PFUnA, PFDoA, PFBS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH) in the blood serum of the US American population are normally less than 1 ng/ml.

Table 5.3: PFAS concentrations in the serum of the general population in the USA from various studies (ATSDR, 2015, p. 10) and mean PoD for PFOA and PFOS (Table 5.2)

Substance	**Concentration** [ng/ml]	**Geometric mean USA**	**Mean PoD from N studies**	
			PoD	**n**
PFOA	2.1 – 9.6	3.9 (Macon *et al.*, 2011)	< 6.6	15
PFOS	14.7 – 55.8	18.4 (Chang, 2009)	< 18.1	11
PFHxS	1.5 – 3.9			
other PFCs	< 1			

For PFOA and PFOS the background concentrations are in the range of the obvious assessment criteria (HBM, 2015a: PFOA-PoD = 2 ng/ml, upper level = 10 ng/ml, PFOS-PoD = 5 ng/ml, upper level = 15 ng/ml). Amplification of the effect due to mixed exposure cannot be ruled out. On the contrary, it can be assumed that the effect of individual PFASs can be amplified through the simultaneous presence of other PFCs. This is shown, for instance, by the study of the Faroe Islands cohort (Grandjean *et al.*, 2012) with co-exposure to PCBs and methyl mercury, which results in a 10-fold lower PoD than all other studies (PFOA: BMD_5 = 0.3 ng/ml serum; PFOS: BMD_5 = 1.3 ng/ml serum).

In three studies on developmental toxicology cited by HBM (2015c, Table 1) no PFOA effect was observed (Table 5.2): Washino *et al.* (2009), Stein *et al.* (2009) and Nolan *et al.* (2009).

∞ Washino *et al.* (2009) did not establish any statistically significant association between birth weight and PFOA concentration in serum in a prospective cohort study with 428 mother-child-pairs (geometric mean of 1.2 ng/ml and spread of < NG up to 5.3 ng/ml). In contrast, a statistically significant association was observed between the markedly higher maternal PFOS concentration in serum (geometric mean of 4.6 ng/ml; spread 1.3 up to 16.2 ng/ml) and birth weight.

∞ Stein *et al.* (2009) examined retrospectively, within the framework of the C8 Health Project, whether associations could be detected between the PFOA/PFOS concentration in the serum of women and endpoints of pregnancies over the last five years (premature birth, birth weight < 2500 g, pre-eclampsia or abnormalities). The sub-group for PFOA measurements refers to participants who had continuously lived in an area with PFOA contaminated drinking water from their pregnancy up to measurement. The geometric mean of the PFOA concentration in the serum is 21.2 ng/ml (spread < NG to 894 ng/ml, n = 1845), the geometric mean for the PFOS serum concentration is 13.6 ng/ml (spread < NG to 83.3 ng/ml, n = 5262). Only in conjunction with PFOS do the authors describe elevated risks of pre-eclampsia (OR[4] = 1.3 for the group above the median compared to the group below the median; 95% confidence range, confidence interval CI, 1.1 up to 1.7). Elevated PFOS concentrations are likewise associated with the risk of lower birth weight. Compared to the reference group within the 50th percentile of the PFOS concentration, the OR for the birth of a child weighing less than 2500 g at birth is 1.5 (95% CI = 1.1 to 1.9) for concentrations above the median, 1.6 (95% CI = 1.1 to 2.3) for concentrations within the 75th – 90th percentile and 1.8 (95%-CI = 1.2 to 2.8) for concentrations above the 90th percentile.

∞ Nolan *et al.* (2009) examined, in a cross-sectional study, 1555 births in North American Washington County, Ohio, for associations between PFOA exposure and birth weight and length of gestation (quoted in Savitz *et al.*, 2012). The PFOA concentration in drinking water is used as the surrogate for PFOA exposure. Four water utility plants provided drinking water:

The mean PFOA measurement values in drinking water were
- for LHWA 6.8 µg/l (spread 1.7 to 17.1 µg/l),
- for Belpre 0.21 µg/l (spread 0.1 to 0.24 µg/l),
- for Marietta 0.0065 µg/l (spread 0 to 0.017 µg/l) and
- for Warren 0.007 µg/l (spread 0 to 0.021 µg/l).

The exposure of the population was divided into three categories:
- high (only supplied by LHWA),
- mean (partially supplied by LHWA) und
- low (not supplied by LHWA).

[4] OR: *odds ratio*

Previous studies have shown that the residents in the area supplied solely by LHWA had, as the median, 80-fold higher PFOA concentrations in the blood (> 300 ng/ml) than the US average. Nolan *et al.* (2009) did not observe any reduction in birth weight or mean gestation duration for the highly exposed cohort compared to the cohorts with mean or low exposure or the general population in the USA. No elevated incidence of premature births or low birth weight (< 2500 g) was observed for this cohort either. The PFOS concentration in the blood of the volunteers is not mentioned. It can be assumed that it was in the normal range in the USA in all the cohorts examined (14.7 – 55.8 ng/ml; ATSDR, 2015). Neither this PFOS concentration nor the PFOA concentration of > 300 ng/ml (Nolan *et al.*, 2009) in the highly contaminated cohort had an impact on the examined parameters of embryonic development.

The multiple exposure in the human epidemiological studies and the lack of an association of PFOA concentrations in serum between 1.2 and > 300 ng/ml with parameters of embryonic development in individual studies (Washino *et al.*, 2009; Stein *et al.*, 2009; Nolan *et al.*; 2009) argues against the general use of the serum concentrations determined in human studies as the PoD for PFOA. Other authors (Bach *et al.*, 2015; Olsen *et al.*, 2009; Verner *et al.*, 2015) did not deem the association between exposure to PFOA and the observed effects to be sufficient or criticised the fact that the glomerular filtration rate, which influences PFAS excretion, is also linked to birth weight (reverse causality).

One interesting goal of the study by Whitworth *et al.* (2012) was the examination of the impact of PFOA and PFOS on the fertility of women who had not yet had any children (nulliparae) because, due to PFC transfer to foetus and breast milk, the body burden of PFCs is reduced during pregnancy and lactation. Whereas in the case of women with earlier births the odds ratio (OR) for the highest PFOA quartile (> 3 ng/ml serum) was 2.2 and 2.1 for the highest PFOS quartile (> 17 ng/ml serum), the corresponding ORs for nulliparae were 0.5 (PFOA) and 0.7 (PFOS). Whitworth *et al.* (2012) argue that the data for the nulliparae could be more informative with regard to the toxic effects of PFCs. If one accepts this line of argument, then no PoD can be derived from this study for the assessment of PFOA and PFOS. The data from the studies by Fei *et al.* (2009) and Vélez *et al.* (2014) are then also challenged when it comes to their suitability as the PoD.

It is, therefore, almost impossible to estimate from the available studies how high the PoD would be in the event of strict mono exposure to a single PFAS.

The best-suited is perhaps the study by Looker *et al.* (2014) with very high PFOA exposure and normal PFOS exposure. Within the framework of the C8 Health Project, these authors examined retrospectively whether they could detect associations between the PFOA/PFOS concentration in the serum of adults and endpoints of humoral immunity. Before and after influenza vaccination, serum samples were taken, analysed for PFOA and PFOS and tested using the haemagglutination inhibition test and for antibodies to influenza viruses. The median values of 411 individuals were 31.5 ng/ml PFOA and 9.2 ng/ml PFOS. The results showed that PFOA was associated with a reduced increase in antibodies to the A/H3N2 influenza virus. The inhibiting effect was clear above the 3rd quartile from 90 ng/ml serum PFOA with a regression coefficient of -0.22 (95 % CI: -0.43 to -0.01). The corresponding PFOS concentration was 14.5 ng/ml and the PFOS concentrations did not correlate with the endpoints examined.

According to equation 6 there is a clear inhibition of antibodies to the A/H3N2 influenza virus at 90 ng/ml:

TDI_{PFOA} = (0.090 mg/l · 0.00835 l/d) / (0.527 · 70 kg) = 0.000 02037 mg/kg·d ≈ 20 ng/kg·d

and the drinking water concentration: 20.37 ng/kg·d · 0.1 · 70 kg · 1 d / 2 l = 71.3 ng/l ≈ 100 ng/l.

The laboratory animal studies continue to be another important basis for a PoD. They likewise indicate a value of around 100 ng/l based on human toxicology (see above). This concentration corresponds to a TDI equivalent of around 30 ng/kg·d (29 ng/kg·d according to Macon *et al.* (2011) and 26 ng/kg·d according to Butenhoff *et al.*, 2002) and a plasma level of 126 ng/ml.

According to ATSDR (2015, p. 22) no studies are available regarding controlled exposures of volunteers to perfluoroalkyl compounds. Health assessments have been conducted of workers exposed to perfluoroalkyls, residents living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and members of the general population presumably exposed to background PFOA levels. The epidemiology studies lack environmental monitoring data; however, most studies used serum perfluoroalkyl levels as a biomarker of exposure. A wide range of effects have been statistically associated with serum perfluoroalkyl levels; however, there is a lack of consistency of the findings across studies and across types of studies. Based on the weight of evidence, there is support for identifying several health effects in humans that appear to be related to perfluoroalkyl exposure: increases in serum lipid levels; increases in uric acid, a possible biomarker for hypertension; small decreases in birth weight; and possible changes in biomarkers of liver damage. The magnitude of the changes in birth weight and serum liver enzymes observed in the human studies are small and probably not biologically relevant).

There was a statistical association between serum cholesterol levels and the serum levels of PFOA (Costa 2004; Costa *et al.* 2009; Eriksen *et al.* 2013; Frisbee *et al.* 2010; Olsen *et al.* 2003a; Sakr *et al.* 2007a, 2007b; Steenland *et al.* 2009b) and of PFOS (Château-Degat *et al.* 2010; Eriksen *et al.* 2013; Frisbee *et al.* 2010; Nelson *et al.* 2010; Olsen *et al.* 1999, 2003a; Steenland *et al.* 2009b) in studies with workers, residents with high PFOA levels in drinking water and the general population. Furthermore, a risk was observed of elevated cholesterol levels and elevated PFOA levels in the serum of adults (Steenland *et al.* 2009b), children and adolescents (Frisbee *et al.* 2010), who lived in an area with elevated PFOA in the drinking water. The risk of elevated cholesterol levels was observed in adults with serum levels of PFOA of 13.2 – 26.5 ng/ml and above and PFOS levels of 13.3 – 19.5 ng/ml and above (Steenland *et al.* 2009b). However, these data are difficult to interpret because the correlation between PFOA and PFOS depends on the level of the serum concentrations. In the case of joint consideration of PFOA and PFOS with serum cholesterol in the same model, an attenuation of 20-30 % was observed (Steenland *et al.* 2009b).

The association between serum PFCs and serum uric acid has been less well examined than in the case of serum lipids. However all five studies that examine this endpoint report statistically significant findings: for instance, between uric acid and PFOA in the serum in workers (Costa *et al.* 2009; Sakr *et al.* 2007b), between uric acid and both PFOA and PFOS in highly exposed residents (Steenland *et al.* 2010b) and in the general

population (Geiger *et al.* 2013; Shankar *et al.* 2011b). The study with the highly exposed residents (Steenland *et al.* 2010b) also observed a significant increase in the risk of hyperuricaemia (> 6 mg/dl for women and > 6.8 mg/dl for men) in individuals with PFOA serum levels of 11.5 - 20.6 ng/ml and above or PFOS serum levels of 17.5 - 23.2 ng/ml and above. In the general population an elevated risk of hyperuricaemia was observed at serum PFOA levels of 3.5 - 5.1 ng/ml or for PFOS levels of 11.2 - 17.8 ng/ml and above (Shankar *et al.* 2011b). In this context the PFOA or PFOS levels only explained less than 1 % of the variance in the uric acid concentration in blood (Steenland *et al.* 2010b).

The two endpoints associated with the serum PFC levels, high cholesterol levels and hyperuricaemia, are the first that would be used as the basis for the derivation of tolerable health values. Given the well-established correlation between serum cholesterol and cardiovascular diseases, this line of argument would be very well supported above all in the case of elevated cholesterol levels. On the other hand, a few studies did not establish any statistically significant association with workers exposed at work (Olsen and Zobel 2007; Olsen *et al.* 2000), highly exposed residents (Emmett *et al.* 2006a; Wang *et al.* 2012) and the general population (Fisher *et al.* 2013) although in eleven studies there were significant correlations between serum PFC values and serum cholesterol levels. In the database there are no studies that measured exposure concentration or exposure dose. In contrast, most of the studies report serum PFC levels as an exposure biomarker. Here, exposure probably took place via several routes. Workers were mainly exposed by the inhalational route whereas, in parallel, the oral route contributed to overall contamination with PFC. Residents living near a PFOA production site were mainly exposed through drinking water but also via the air route to PFCs. One study with residents living close to industrial plants that handled PFOA (Emmett *et al.* 2006a), scarcely established any difference between residents with probable minimal exposure to airborne PFOA (mean serum level of 418 ng/ml) and those with higher exposure to airborne PFOA (mean serum level also 418 ng/ml). In this case, most, if not all, the individuals were exposed to several PFCs. Studies with highly exposed residents and the general population frequently report a significant association for both PFOA and PFOS. There are no known reports of possible confounding of the various PFCs with the relevant health effects (ATSDR, 2015). What further complicates this situation is the fact that the mechanisms of toxic effects are not known nor are the mechanisms from animal experiments either. It is the case that serum levels of cholesterol and other lipids are also affected in rats and mice; exposure to PFCs in rodents, however, leads to a reduction in the serum lipid levels (end of citation).

The ATSDR (2015) rules out the use of the known epidemiological studies as the basis for the quantitative assessment of PFOA (and PFOS) because of the uncertainties outlined by them as indicated above.

Budtz-Jørgensen, Keiding and Grandjean (2001) come to a similar conclusion: "*A threshold for dose-dependent toxicity is crucial for standards setting but may not be possible to specify from empirical* [epidemiological] *studies*." Also the recently published PFOA assessment by the American Environmental Protection Agency (US-EPA, 2016) views the human epidemiological studies merely as a qualitative criterion for hazard identification and as support for animal experiment findings. This is because the serum levels, at which the examined effects first occurred, could not be determined and it is not known whether a steady state had been achieved when the effects occurred. The

problems are further exacerbated by possible PFOA precursors which may already have had an impact. Other disruptive factors were a series of PFAS and other contaminants in the blood of the volunteers examined.

The quantitative ratio between the lower PFOA level and the higher PFOS level in blood serum according to E-HBM (2016; PFOA: PFOS = 2 ng/ml: 5 ng/ml) is not confirmed by the data from animal experiments. It is not possible to derive such a clear stronger efficacy of PFOA versus PFOS from these data.

The data presented in Table 5.3 for PFOA in the serum of the general population in the USA, which seemed to be lower than for PFOS, possibly reflect more the global ubiquitous exposure. Ubiquitous exposure to PFCs, as a substance group of what are known as persistent organic pollutants (POPs), is also observed in the studies conducted in dead peregrine falcon eggs in Baden-Württemberg (Schwarz *et al.*, 2016; v. d. Trenck, 2014; v. d. Trenck, 2012). This is likewise confirmed in the study by Lindh *et al.* (2012) on PFC serum concentrations in Greenland, Poland, (roughly comparable with Germany) and the Ukraine. This study identified median (and mean) values [in ng/ml] for PFOA of 4.5 (4.8), *4.8 (5.3)* and 1.3 (1.8) and for PFOS of 44.7 (51.9), *18.5 (18.6)* and 7.6 (8.1).

The derivation of a tolerable dose on the basis of corresponding data from human epidemiological studies (HBM, 2015a) then results in a similar ratio between PFOA and PFOS. This kind of scepticism about this assessment basis is confirmed to a certain extent by Corsini *et al.* (2012): these authors have tested a number of PFCs with regard to their immunosuppressant action in human cells *in vitro* and have come to the conclusion: "... PFOA is the PFC with the least pronounced action followed by PFBS, PFDA, PFOS, PFOSA and fluorotelomers."

Proposal for a DW_{GV}

The results of the various derivation routes are presented in Table 5.4.

The animal studies lead to a value of 100 ng/l based on human toxicology. This value corresponds to a TDI equivalent value of around 30 ng/kg·d (29 ng/kg·d according to Macon *et al.*, 2011, or 26 ng/kg·d according to Butenhoff *et al.*, 2002) and a plasma level of 126 ng/ml.

A corresponding value from the epidemiological study by Looker *et al.* (2014) is just slightly lower. In contrast, the assessment results of the HBM Commission (HBM, 2015a, E-HBM, 2016) are far lower. Against the backdrop of the problems of multiple exposure in epidemiological studies discussed above and the doubtful so clearly different efficacy of PFOA and PFOS, a tolerable drinking water concentration of 100 ng/l for PFOA seems plausible.

Hence, a DW_{GV} of 100 ng/l is proposed for PFOA.

By way of deviation from the assessment by the HBM Commission, the same level is given for PFOA and PFOS. This corresponds to the procedure of the US-EPA (2016) which gives a slightly lower value of 70 ng/l respectively (based on a reference dose of 20 ng/kg·d, US-EPA, 2016a, b) for both PFOA and PFOS.

Table 5.4: Assessment results for PFOA from various studies and resulting values

Study	Serum conc. [µg/l] or conversion to a serum concentration in steady state (Css) according to HBM, 2015d	according to ATSDR, 2015	according to Post *et al.*, 2012	TDI (Dss) [µg/(kg·d)]	TW conc. (C_{TW}) [µg/L]
26-w monkeys (Butenhoff *et al.*, 2002)	Css = **114** (with Dss = TDI) control ≈ **203**[1)]	Css = **260** (with Dss = TDI) control ≈ **203**[1)]	C_{serum} = **9.0** (with C_{TW} = 89.8) control ≈ **203**[1)]	$BMDL_{10}$ / EF = 1.54 / 60 = **0.025 7**	**0.090**
Pregnant mice (2nd phase – 8-17 d) (Macon *et al.*, 2011)	Css = **126** (with Dss = TDI) **284.5**[2)]	Css = **289** (with Dss = TDI) **284.5**[2)]	C_{serum} = **10** (with C_{TW} = 100) **284.5**[2)]	LOAEL / EF = 10 / 350 = **0.028 57**	**0.1**
Human epidemiology, far lower vaccine protection after influenza vaccination (Looker *et al.*, 2014)	Css = **90**	→		Dss = **0.020 37** (with Css = 90)	**0.071**
		Css = **90**	→	Dss = **8.9** (with Css = 90)	**0.031**
			C_{serum} (= C_{ss}) = **90**	→	**0.9** (with Css = 90)
Human epidemiology, upper limit (HBM, 2015a)	Css = 10	→		Dss = **0.002 3** (with Css = 10)	0.008
Human epidemiology (E-HBM, 2016)	Css = 2	→		Dss = **0.000 45** (with Css = 2)	0.002
Estimated value for PFOA bearing in mind mixed exposure in epidemiological studies	**126** (with Dss = TDI)	←		**0.028 6**	**0.1**

Calculation bases

Source	HBM, 2015d	ATSDR, 2015; USEPA, 2016a with $t_{1/2}$ = 840 d	Post *et al.*, 2012
Serum **C_{ss}** formula; steady state concentration (C_{ss}) in the serum	$Css = \frac{Dss \cdot R \cdot 70\ kg}{F} = Dss \cdot 4418\ \frac{kg \cdot d}{l}$	$Css = \frac{Dss \cdot AF}{ke \cdot Vd} = Dss \cdot 10\,101\ \frac{kg \cdot d}{l}$	Estimated $C_{serum} = 100 \cdot C_{TW}$ (If C_{TW} = 0.1 µg/L, then C_{serum} = 10 µg/L)
Dose **Dss** (resulting daily intake level)	$Dss = \frac{Css \cdot F}{R \cdot 70\ kg} = Css \cdot 0.000\,226\,3\ \frac{l}{kg \cdot d}$	$Dss = \frac{Css \cdot ke \cdot Vd}{AF} = Css \cdot 0.000\,099\ \frac{l}{kg \cdot d}$	
Parameter	F (D_{ss}/C_{ss}) = 0.00835 l/d R (time correction) = 0.527	k_e = 0.693/1400 d = 0.000 495 d^{-1} V_d = 0.2 l/kg AF (absorption factor) = 1	

[1)]: Mean control: 203 ng/ml; spread range: <LoQ – 230 ng/ml (Butenhoff *et al.*, 2002)

[2)]: 284.5 ng/ml = PFOA serum concentration of the LOAEL, measured the day after the birth of the female mice (Suppl. Table 7 in Macon *et al.*, 2011)

It should, however, be borne in mind that human biomonitoring considers the actual exposure situation in a manner which is closer to reality than consideration of an individual substance. It is, therefore, the preferred assessment instrument. If it is possible to do human biomonitoring, then of course the HBM I-value is deemed to be a comparative benchmark.

If only drinking water levels are available, then the addition rule in accordance with TRGS 402 is to be applied in the event of the parallel occurrence of several PFCs (BAuA, 2010; EU, 2012; LAWA, 2010). The assumed additive effect of the regulated PFCs results in a far stricter assessment than if each individual substance were only assessed in isolation. This applies in particular when half of LoQ is used for erroneous findings (< LoQ). However, this, too, corresponds to the procedure of the US-EPA, which also gives a value of 70 ng/l in the event of the joint occurrence of PFOA and PFOS (EPA, 2016b, result equivalent to the addition formula for PFOA and PFOS). Reference is also made at this point to the fact that, with this addition rule, the rounding of initial levels (PoD) from the epidemiological study by Looker *et al.* (2014) would to a certain degree be compensated.

Quantitative human toxicological assessments by other institutions

The German Environment Agency (UBA, 2011) derived three close TDI equivalent values after consideration of the known dose-effect relationships for the sum of PFOA and PFOS: based on a study in rats with total extrapolation factor, EF, 300: 0.08 µg/kg·d, based on a study in monkeys with an EF of 900: 0.15 µg/kg·d and based on an MAC value (maximum allowable concentration at the workplace) with an EF of 10: 0.06 µg/kg·d. It recommends using a rounded value of 0.1 µg/kg·d in practice (Dieter, 2007; BfR, 2006; TWK, 2006). With the customary conventions this results in a lifelong tolerable level based on human toxicology of 0.3 µg/l drinking water.

In parallel to this the Drinking Water Commission suggests a value of 0.1 µg/l as the minimum quality goal for lifelong preventive health care. Drinking water with more than 0.5 µg/l (Σ PFOA + PFOS) should not be used to prepare baby formula (Schulte, 2006; Dieter, 2009; UBA, 2011). For the assessment of mixtures of carboxylic acid and sulfonic acid with three to eight perfluorinated carbon atoms in drinking water, the German Environment Agency makes a proposal which is based on three drinking water guideline values (GVs) and seven health-related indication values (range of values between 0.3 and 7 µg/l; Lud *et al.*, 2010).

When deriving test values for the contamination routes soil-man and soil-groundwater, the LUBW (2014) had to choose between TDIs, which came from the fields of drinking water or food surveillance. German food surveillance (chemical testing agencies) works with the EFSA values of 0.15 for PFOS and 1.5 µg/kg·d for PFOA (EFSA, 2008). However, they do not take into account the very varied excretion rates of rats and humans and, in the case of PFOA, they base this on a dose which is still in the range of measurable effects [$BMDL_{5\text{-}10}$ = 0.3 mg/kg·d, CSR, 2009]. Hence, for the test values derived by the LUBW (2014) a TDI of 0.1 µg/kg·d was taken as the base for PFOS and PFOA and for the sum of

the two substances taken from Dieter (2007) and the UBA (2011), and which was agreed with BfR (2006) and the Drinking Water Commission (TWK, 2006). This decision was not challenged either by the plenary of the Symposium "Perfluoroalkylated substances (PFAS): Status quo of health assessment" (BfR, 2014). The epidemiological research findings summed up by Schümann at this symposium are also grounds for rethinking the EFSA TDIs. According to them an effect threshold is not to be assumed in humans. That is why this author suggests an updated risk assessment for PFOS and PFOA (Schümann, 2014).

The Office of Water of the U.S. EPA (EPA, 2016c) established a reference dose (RfD) of 0.02 µg/kg·d from the study on developmental toxicology in mice by Lau *et al.* (2006). The reference dose is based on reduced ossification and accelerated puberty (in males). It was determined by attributing a total factor 300 (factor 10 for the determination of a NOAEL from a LOAEL, factor 3 for the toxicodynamic differences between mice and humans and factor 10 for consideration of sensitive humans) to a human equivalent LOAEL (HED_{LOAEL}). This reference dose would result in a drinking water concentration of 70 ng/l using the customary measurements.

The Canadian health authority has prepared a proposal for a maximum admissible drinking water concentration of 0.2 µg/l of PFOA, which is currently in the phase of public commentary (Health Canada, 2016). This is based on a TDI of 25 ng/kg·d, derived from non-carcinogenic effects in animal experiments, which was low enough in order to protect against the carcinogenic effects of PFOA as well.

According to the Canadian health authority epidemiological studies have also demonstrated the association between PFOA exposure and various non-carcinogenic health effects (like disruption of the immune system and changes in birth weight and lipid values). However, because of various constraints, these studies could not be used to derive a TDI (Health Canada, 2016). In animal experiments reproduction and development disorders, non-carcinogenic liver effects and abnormal serum lipid levels were observed. Liver cell hypertrophy in rats is deemed to be the most suitable endpoint for the derivation of a TDI, which occurs at the same dose as changes to serum lipids.

The point of departure is a NOAEL of 60 µg/kg·d for hepatocellular hypertrophy in male rats from the pivotal study by Perkins *et al.* (2004). This is extrapolated to humans with a dose-specific kinetic factor 96 and a dynamic factor 2.5. In addition there is an intraspecies factor 10 to allow for variation in humans. This results in a TDI of 0.025 µg/kg·d. With drinking water consumption of 1.5 l/d, body weight of 70 kg and a TDI allocation to drinking water of 20 %, this results in a tolerable drinking water concentration of ($0.025 \cdot 70 \cdot 0.2 / 1.5 = 0.23$ µg/l) approximately 0.2 µg/l. Hence, the Canadian assessment does not differ in terms of the TDI. It differs only from the DW_{GV} recommended here because of the assumptions about the drinking water quota and drinking water consumption.

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6 Perfluorononanoic acid, PFNA (375-95-1)

ATSDR (2009), RAC (2014), Post (2014), Bull *et al.* (2014) and NJDWQI (2015) give overviews of the human toxicity of PFNA.

Tatum-Gibbs *et al.* (2011) and Ohmori *et al.* (2003) report elimination half-lives of 30.6 and 29.6 days for male and 1.4 and 2.4 days for female rats and Tatum-Gibbs *et al.* (2011) 34.3 - 68.9 days for male and 25.8 - 68.4 days for female mice. For elimination half-lives in humans Zhang *et al.* (2013) determined an arithmetic mean of 4.3 years (min. 0.34 years, max. 20 years) for a cohort of 50 men and older women. Younger women had a significantly shorter elimination half-life (arithmetic mean 2.5 years, min. 0.38 years, max. 7.7 years; n = 16).

Das *et al.* (2015) conducted a study on developmental toxicity. From their results they derived benchmark doses (BMD_5 and $BMDL_5$) for several toxicological endpoints. For this, PFNA doses of 1, 3, 5 or 10 mg/kg were administered daily by oral gavage to CD-1 mice on gestation days 1-17; furthermore, a control group was given deionised water. Dams given 10 mg/kg·d could not carry their offspring to term and were not examined further. PFNA caused hepatomegaly (liver enlargement) in the gestating dams at a dose of 5 mg/kg·d or lower doses but it had no effect on the number of implantations, foetal viability or foetal weight. The offspring of the groups given 1 and 3 mg/kg·d were born alive and post-natal survival corresponded to that of the control group. 80 % of the living born offspring of the group with a dose of 5 mg/kg·d died within the first ten days. Das *et al.* (2015) report the following lowest $BMDL_5$ (BMD_5):

- absolute dam liver weight on gestation day 17 elevated: 0.27 mg/kg·d (0.43 mg/kg·d),
- relative dam liver weight on gestation day 17 elevated: 0.21 mg/kg·d (0.30 mg/kg·d),
- relative foetal liver weight on gestation day 17 elevated: 0.22 mg/kg·d (0.36 mg/kg·d),

- elevated relative liver weight of offspring on day 1 after birth 0.19 mg/kg·d (0.24 mg/kg·d).

According to the EU Committee for Risk Assessment (RAC) "proposing harmonised classification and labelling at EU level of perfluorononan-1-oic acid (PFNA) and its sodium and ammonium salts" seem to sufficiently meet the criteria for a read-across method on the basis of the high structural similarity and chemical analogies for PFNA (RAC, 2014). Hence the following recommendation is made: to classify PFNA (and its sodium and ammonium salts) as a carcinogen 2 (labelling H351, suspected of causing cancer) and Repr. 1B (H360Df, may damage the unborn child and is suspected of damaging fertility.

PFNA did not demonstrate any effects in a study with human mammary adenocarcinoma cells MCF-7 that examined oestrogen-like potential *in vitro* (*inter alia* with analyses of cell cycle and gene expression of oestrogen-response-biomarker-genes) (Maras *et al.*, 2006).

Wielsøe *et al.* (2015) examined a number of PFCs *in vitro* with regard to the triggering of oxidative stress and DNA damage in the human liver cell line HepG2 in concentrations of $2 \cdot 10^{-7}$ to $2 \cdot 10^{-4}$ M (oxidative stress) or $2 \cdot 10^{-5}$ M (DNA damage). PFNA increased the formation of reactive oxygen species (ROS), and DNA damage in the Comet assay. The DNA damage (indicated as a mean percentile of tail intensity) increased more than three-fold at the highest concentration compared with the solvent control.

Eriksen *et al.* (2010) examined the genotoxic potential of PFNA *in vitro* in human HepG2-cells (together with PFBS, PFHxA, PFOA and PFOS). It aimed to evaluate the potential for the formation of reactive oxygen species (with PFNA concentrations of 0.4, 4, 40, 200, 400, 1.000 and 2.000 µM) as a cause of DNA damage (examined separately with PFNA concentrations of 100 µM and 400 µM). With both concentrations PFNA caused a moderate but statistically significant increase in DNA strand breaks (1.6-fold higher than the control, 95 % confidence interval 1.09–2.12). This effect was not linked to the formation of reactive oxygen species. The finding was attributed to cytotoxic concentrations, whereby this point remains unclear as no cytotoxicity occurred at 100 µM PFNA (Post, 2014).

Wolf *et al.* (2010) proved in mice that the peroxisome proliferator-activated receptor PPARα is a main mediator in developmental toxicity induced by PFNA. In COS-1 cells, into which PPARα plasmids of mice or humans were transfixed, 0.5-100 µM PFNA activated the luciferase of plasmids of both mice and humans in comparison to the controls in a concentration-dependent manner. The PPARα of mice reacted to PFNA more than twice as sensitively as human PPARα; out of the eight up to 13 tested PFCs, PFNA showed the strongest effect (Wolf *et al.*, 2008, 2012). With PPARα mice plasmids concentration dependence was demonstrated up to 256 µM; binary mixtures of PFNA and PFOA had an additive effect at low concentrations (Wolf *et al.*, 2014). Buhrke *et al.* (2013) confirmed the activation of human PPARα by PFNA; the effect of PFNA (and the longer chain PFDA and PFDoA) was lower than that of PFHxA, PFHpA and in particular PFOA.

Kudo *et al.* (2000) examined the strength of the induction of peroxisome β-oxidation by various PFCs *in vivo* that is closely related to PPARα activity (Post, 2014). Out of the C6 to C9 PFCs, PFNA demonstrated the strongest effects (β-oxidation and liver enlargement increased with length of chain, Kudo *et al.*, 2006).

In the steroidogenesis test PFNA, with a human adrenocortical carcinoma cell line (NCI-H295R), did not lead to the formation of 17β-oestradiol or testosterone and did not react either in the reporter gene tests with human androgen or Ah-receptors. In the oestrogen receptor assay, increased activity was measured coupled with elevated variability (share of living cells; Rosenmai *et al.*, 2014).

In the oestrogen receptor (ER) transactivation assay, PFNA (just like the longer chain PFDA, PFUnA, and PFDoA) showed no oestrogenic activity in the oestrogen-sensitive genetically transformed mammary carcinoma cells (MVLN-cells). In the androgen receptor (AR) transactivation assay, PFNA (like PFHxS, PFOA, PFOS and PFDA) acted as a concentration-dependent antagonist (Kjeldsen and Bonefeld-Jørgensen, 2013).

RAC (2014) describes studies on PFNA with "S-111-S-WB". Here S-111-S-WB (CAS No. 72968-38-8, also "Surflon S-111") is a mixture of perfluorinated acid ammonium salts of various chain lengths. It is used because of its surface activity in polymer production. The CAS No. stands for ammonium salts from C7 to C13 perfluorocarbolyxic acids. The mixture contains 70–80 % ammonium perfluorononanoate (C9), 15-20 % ammonium perfluoroundecanoate (C11), 5 % ammonium perfluorotridecanoate (C13) and < 1 % ammonium octanoate (van the Putte *et al.*, 2010).

S-111-S-WB was administered by oral gavage to ten Charles-River-CD(SD) rats for each dose and gender at doses of 0.025, 0.125, and 0.6 mg/kg·d over 90 days (Mertens *et al.*, 2010). For this study the New Jersey Drinking Water Quality Institute (NJDWQI, 2015) refers to Prevedouros *et al.* (2006) as the source for the composition of the mixture and gives 74 % PFNA, 20 % PFUnA (C11), 5 % PFTriA(C13), 0.78 % PFOA (C8), 0.37 % PFDA (C10), and 0.1 % PFDoA (C12). The study identified elevated liver weights with hepatocellular hypertrophy and elevated hepatic β-oxidation at 0.125 and 0.6 mg/kg·d. The males given a dose of 0.6 mg/kg·d developed hepatocellular degenerations and necroses and demonstrated reduced serum protein and elevated bilirubin and BUN (blood-urea-nitrogen). In both genders the dose of 0.6 mg/kg·d reduced globulin and increased alkaline phosphatase, in the males already at 0.125 mg/kg·d. The authors give 0.025 mg/kg·d as the NOEL for males and 0.125 mg/kg·d for the females.

Stump *et al.* (2008) conducted a 2-generation study with Charles-River-CD(SD) rats with S-111-S-WB. The dose for 30 animals per gender and dose group (0, 0.025, 0.125 or 0.6 mg/kg·d) was the same as in the study by Mertens *et al.* (2010). According to the presentation by the NJDWQI (2015) this also applies to the mixture composition. The F_0 generation was administered a dose by oral gavage at least 70 days prior to mating, during mating, gestation and lactation up to necropsy. The total duration is not reported. Based on the charts the NJDWQI (2015) concludes a dosage period of 18 weeks. For the F_1 generation the same applied in principle. In turn, the NJDWQI (2015) reads from the

graphic chart that the dose started at an age of around four to six weeks and continued over 21 weeks.

The exposures did not demonstrate any impact on litter size, survival rate of offspring or body weight. In the high dose group a reduced mean body weight was observed in male F_0 and F_1 generations. In this dose group there were also higher liver weights, correlated with microscopic findings of hepatocellular hypertrophy, in the males of the F_0 generation also in the mean dose group and for two dose groups also in the F_1 generation. In the two higher dose groups higher kidney weights were observed in the F_0 generation, in the high dose group coupled with hypertrophy of kidney tubules. The authors select the highest dose of 0.6 mg/kg·d as the NOAEL for the reprotoxic effect and a dose of less than 0.025 mg/kg·d on the basis of microscopic findings in the liver of the males as the systemic toxicological NOAEL for the F_0 and F_1 generations. In the case of foetal toxic effects, they select an NOAEL of 0.025 mg/kg·d because of elevated liver weight in the F_1 and F_2 offspring at the next higher dose.

Rationale of the DW_{GV}

With the 2-generation study by Stump *et al.* (2008) and the 90-day study by Mertens *et al.* (2010) there are sufficient toxicological data of relevance for assessment for the rationale of a DW_{GV} although it is problematic that they were not conducted with PFNA but with the mixture S-111-S-WB.

The 2-generation study by Stump *et al.* (2008) is deemed to be the basis of the highest quality. The following results from the NOAEL "smaller" 0.025 µg/(kg·d) reported there: factor 3 is used to extrapolate to a "safe" NOAEL and factor 50 for interspecies variability in toxicokinetics (elimination half-life male rats approximately 30 days, human approximately 1570 days ≈ 50). Given the greater sensitivity of rodents for the relevant effect (peroxisome proliferative liver enlargement at the mean dose, parallel higher relative kidney weight without numerical and/or statistically significant increase at higher doses), no factor is given for interspecies variability in toxicodynamics. A factor for intraspecies variability does not seem necessary because of a 2-generation study in which there was exposure already during the prenatal phase and during lactation. Based on the rationale of the RAC (2014) for classification as possibly carcinogenic (Carc. 2), supported by the classification as reprotoxic (Repr. 1B) and the findings on *in vitro* genotoxicity, an additional safety factor 10 is to be included. This results in a total factor 1500. This leads to the calculation of tolerable human intake of 16.7 ng/kg·d. With the customary basic parameters (70 kg body weight, 2 litres drinking water consumption per day, 10 % allocation), this results in a DW_{GV} of (58.3 or rounded) 60 ng/l.

A derivation based on the study by Mertens *et al.* (2010) would require far higher overall extrapolation and a safety factor (around 50,000 with factor 10 for time extrapolation, 50 for interspecies variability in toxicokinetics – elimination half-lives male rats approx. 30 days, humans approx. 1570 days ≈ 50, factor 1 for interspecies variability in toxicodynamics, factor 10 for intraspecies variability and safety factor 10 for suspected carcinogenicity). This also applies if the reprotoxicity study (exposure time 16 days) by

Das *et al.* (2015) and the $BMDL_5$ reported there were taken as the basis (total extrapolation factor > 30,000, factor 30 for interspecies variability toxicokinetics - elimination half-lives, female mice approx. 50 days, humans approx. 1570 days ≈ 30, factor 1 for the toxicodynamics, intraspecies variability 10, factor > 10 for time extrapolation and safety factor 10 for suspected carcinogenicity). Hence, assessments of this kind based on these studies would have to be deemed to be extremely unreliable.

Quantitative human toxicological assessments by other institutions

The New Jersey Department of Environmental Protection presented for PFNA the draft of a provisional groundwater criterion for chronic (lifelong) drinking water use (Post, 2014). This criterion is based on benchmark modelling for the PFNA blood serum level which caused elevated liver weights in mice (20-25 per group) (Lau *et al.*, 2009, Lau 2014) after 16-day exposure (1, 3, or 5 mg/kg·d and group on gestation days 1-17). The benchmark calculation with the data by Lau *et al.* (2009) and Lau (2014) resulted in 5.2 µg/ml for the $BMDL_{10}$ as the point of departure. Furthermore, factors for the extrapolation from laboratory animals to humans (no factor for toxicokinetics, as the derivation is based on data for the blood serum, factor 3 for toxicodynamics), for consideration of sensitive population groups (factor 10) and for transfer to chronic exposure, and a safety factor because of gaps in toxicological data (together factor 10) were included. Based on toxicokinetic data of laboratory animals and humans, a factor was estimated for the ratio of PFNA intake to PFNA blood serum level of 0.085 ng/kg·d per ng/l. This would correspond to a ratio of blood serum to drinking water of 200:1 (central estimation) for humans with ongoing drinking water consumption.

This resulted in 2015 in the New Jersey Drinking Water Quality Institute indicating a Health-based Maximum Contaminant Level of (12.5 ng/l or rounded) 13 ng/l (0.013 µg/l) (NJDWQI, 2015).

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7 Perfluorodecanoic acid, PFDA (335-76-2)

ATSDR (2009), Bull *et al.* (2014) and SCA (2015) give overviews of the human toxicological data on PFDA.

After the single injection of 48.64 mmol/kg body weight the half-life for Wister rats was 39.92 ± 8.62 days for males and 58.57 ± /5.84 days for females. The difference between the genders was statistically significant (Ohmori *et al.*, 2003).

In the *in vitro* genotoxicity test (with the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA 1538, with a concentration of 5 µmol/plate and with/without metabolic activation), no statistically significant increase in the revertant counts was observed in two independent studies (Buhrke *et al.*, 2013).

In vitro studies on the effect of PFDA (0.1-10 µg/ml) on cytokines in human peripheral blood lymphocytes and human leukaemia cells (THP-1) in the presence of lipopolysaccharides or phytohaemagglutinin demonstrated the inhibition of the tumour necrosis factor-α, interleukine-6 and -10 and interferon-γ. In THP-1 cells PFDA also inhibited NF-κB activation (a specific transcription factor) and prevented I-κB degradation (I-κB is an enzyme complex that is part of the NF-κB transduction cascade), but it did not activate the peroxisome proliferator-activated receptor α (PPARα; Corsini *et al.*, 2012).

Kudo *et al.* (2000) examined the degree of induction of peroxisome β-oxidation by various PFCs *in vivo* in the liver of male and female rats. The effect of PFDA was weak in the males but strong in the females. In cultured hepatocytes of male and female rats the induction of peroxisomal β-oxidation was, in contrast, equally high.

In COS-1 cells, into which PPARα plasmids of mice or humans were transfixed, 0.5-100 µM PFDA activated the luciferase of mouse plasmids in comparison to the controls in a concentration-dependent manner. Out of 13 tested PFCs PFDA showed activity in the mean range. PFDA was the only compound that was inactive in human plasmids (Wolf *et al.*, 2008, 2012). According to Buhrke *et al.* (2013) human PPARs are also activated by PFDA, even if this is the least pronounced (compared with the shorter-chain PFBA, PFHxA, PFHpA, PFOA or PFNA).

Wielsøe *et al.* (2015) examined a number of PFCs *in vitro* with regard to the triggering of oxidative stress and DNA damage in the human liver cell line HepG2 at concentrations of $2 \cdot 10^{-7}$ to $2 \cdot 10^{-4}$ M (oxidative stress) or $2 \cdot 10^{-5}$ M (DNA damage). PFDA increased the

formation of reactive oxygen species (ROS); the DNA damage in the Comet assay was limited (not significant).

In the steroidogenesis assay PFDA (like PFUnA and PFDoA) induced, with a human adrenocortical carcinoma cell line (NCI-H295R), the formation of 17β-oestradiol; progesterone and testosterone levels were not affected. PFDA did not react either in the reporter gene tests with human androgen or Ah receptors (Rosenmai *et al.*, 2014).

In the oestrogen receptor (ER) transactivation assay PFDA (like the longer chain PFUnA and PFDoA as well as PFNA) did not show any oestrogen activity in oestrogen-sensitive genetically transformed mammary carcinoma cells (MVLN cells). In the androgen receptor (AR) transactivation assay PFDA (like PFHxS, PFOA, PFOS and PFNA) acted, depending on the concentration, as an antagonist (Kjeldsen and Bonefeld-Jørgensen, 2013).

In studies on reproduction toxicity following the administration of PFDA by oral gavage in female C57BL/6N mice (10-14 animals per group) on gestation days 6–15 (0.03, 0.3, 1, 3, 6.4, 12.8 mg/kg·d), a significantly reduced body weight at12.8 mg/kg·d compared to the controls was observed in the dams on gestation day 18 and significantly elevated relative liver weight of 1 mg/kg·d. In terms of reproduction toxicity there was an elevated rate of resorption per litter of 19.1 % at 6.4 mg/kg·d and of 41.7 % at 12.8 mg/kg·d, and significantly reduced foetal body weight dose-dependent from 1 mg/kg·d. Compared to the control (mean 7.1 ± 0.3) there was a lower number of live foetuses at 6.4 mg/kg·d (5.8 ± 0.7, not significant) and at 12.8 mg/kg·d (2.6 ± 1.0, significant; Harris and Birnbaum, 1989).

The Proposal for Harmonised Classification and Labelling (CLH report) of the Swedish Chemicals Agency on Nonadecafluorodecanoic Acid (PFDA) and its ammonium and sodium salts suggests, in line with a read-across method, classification (and labelling) as Carc. 2 (labelling H351, suspected of causing cancer) and Repr. 1B (H360Df, may damage the unborn child, is suspected of damaging fertility; SCA, 2015).

Rationale of the health-related indication value

No studies with sub-chronic or longer oral exposure of relevance for the assessment are available. Hence, it is not possible to derive a DW_{GV} based on human toxicology.

According to the health-related indication value concept (Grummt *et al.*, 2013, UBA, 2003), this leads for PFDA in line with the data situation (*in vitro* genotoxicity negative, *in vitro* immunotoxicity positive) to a health-related indication value of 0.1-0.3 µg/l. On the basis of the classification proposal as possibly carcinogenic, backed by the proposal for classification as reprotoxic in category Repr. 1B, a health-related indication value of 0.1 µg/L is recommended.

Quantitative human toxicological assessments by other institutions

The ATSDR (2009) notes that the data available on PFDA are not sufficient in order to derive a minimal risk level.

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8 Perfluorobutane sulfonic acid, PFBS (375-73-5)

The *in vitro* examination of the induction of oxidative DNA damage and the potential for the formation of reactive oxygen species (ROS) in human hepatoma cells HepG2 from up to 2000 µM PFBS did not present any effects in comparison to the controls (Eriksen *et al.*, 2010).

A study of the pharmacokinetics of potassium-PFBS (K-PFBS) in rats, monkeys and humans examined clearance after oral and intravenous (i.v.) administration in rats and after i.v. administration in macaque monkeys (*Macacus cynomolgus*, n = 3 per gender) and the serum half-lives in five men and one woman, who were exposed at their workplace. The PFBS concentrations were measured in serum and urine and in rats also in liver and faeces. In rats the mean elimination half-life after i.v. administration of 30 mg/kg K-PFBS was 4.51 ± 2.22 h in the males and 3.96 ± 0.21 h in the females; after oral administration of the same dose 4.68 ± 0.43 h (males) and 7.42 ± 0.79 h (females). At a low single PFBS dose the mean half-life also falls (males 2.1 h, females 0.64 h after the i.v. administration of 10 mg/kg, confidence intervals not given; Chengelis *et al.*, 2009). In monkeys the corresponding values after the i.v. administration of 10 mg/kg PFBS were 95.2 ± 27.1 h in the males and 83.2 ± 41.9 h in the females. Although the differences in the elimination half-lives in the male and female rates were not statistically significant, clearance in the female rates was significantly higher (469 ± 40 ml/h) than in the males (119 ± 34 ml/h) and the area under the curve (AUC) was significantly higher for male (294 ± 77 µg·h/ml) than for the female rats (65 ± 5 µg·h/ml). These gender-specific differences were not observed in monkeys. The serum half-life for the five men and one woman, who were exposed at their workplaces, was up to 180 days, the geometric mean was 25.8 days (95 % confidence interval 16.6 - 40.2 days; Olsen *et al.*, 2009).

In rats an oral gavage study was conducted with K-PFBS over 90 days. Doses of 60, 200, and 600 mg/kg·d K-PFBS were administered. In addition to clinical observations, feed consumption and body weight were registered as toxicological endpoints and macro- and microscopic pathological, clinical chemistry and haematological tests conducted. In the 60 and 200 mg/kg·d dose groups; nasal cavity, nasal concha, stomach and kidneys were also examined histologically. The K-PFBS administration did not demonstrate any effect on mortality, body weight or neurological parameters. Erythrocyte count (in the 600 mg/kg·d-group), haemoglobin and haematocrit values were significantly reduced (haemoglobin in g/dl: control: 16.4 ± 0.96; 60 mg/kg·d: 16.0 ± 0.41; 200 mg/kg·d: 15.6 ± 0.48, $p \leq 0.05$; 600 mg/kg·d: 15.5 ± 0.78, $p \leq 0.05$; haematocrit values in %: control: 44.2 ± 2.32; 60 mg/kg·d: 42.7 ± 1.44; 200 mg/kg·d: 41.9 ± 1.50, $p \leq 0.05$; 600 mg/kg·d: 40.9 ± 2.24, $p \leq 0.01$) in the males at doses of 200 or 600 mg/kg·d. The female rats in this study did not present any significant effects up to the highest dose of 600 mg/kg·d. For the male rats the NOAEL was 60 mg/kg·d because of the haematological effects at the next higher dose (200 mg/kg·d; Lieder *et al.*, 2009a).

A two-generation study in rats was also conducted with K-PFBS. 30, 100, 300 and 1000 mg/kg·d K-PFBS were administered by oral gavage over a period of ten weeks before and during mating as well as during gestation and lactation (females) to the males and

females of the parental generation (P). The first filial generation (F_1) was given the same dose after weaning. The second filial generation (F_2) was no longer directly exposed and the study was terminated three weeks after their birth. Body weight was registered and feed consumption, clinical symptoms, oestrus cycle, sperm quality, pregnancy, birth, litter size and development parameters were examined. No adverse effect was detected either in the P or in the F_1 generations at a dose of 100 mg/kg·d (NOAEL). In the dose groups with 300 and 1000 mg/kg·d elevated liver weights (absolute and relative) were observed in the males and, correspondingly, increased occurrence of adaptive hepatocellular hypertrophy and in males and females the increased occurrence of minimal up to minor microscopic findings in the renal medulla and renal papilla. No K-PFBS-mediated (biologically relevant) effects were observed on fertility, reproduction or the corresponding parameters either in the P or in the F_1 generations. In the two-generation study there were no K-PFBS-mediated effects on the survival rate of offspring. Litter size and average birth weight per litter were not statistically significant from the controls in any dose group. In the F_1 generation the final body weight in the males of the group at 1000 mg/kg·d was reduced and preputial separation at this dose which was delayed by two days was possibly linked to this. In the F_1 females no major effects were observed, the F_2 offspring had normal body weights. According to the authors (Lieder *et al.*, 2009b) the reproductive NOAEL in both generations was > 1000 mg/kg·d.

When male mice were given a daily dose of K-PFBS in feed over a period of 4-6 weeks, a reduction in plasmatriglyceride (- 37 %) and of non-HDL lipoprotein (non-high-density lipoprotein; - 28 %) were observed along with the elevated clearance of (radioactive labelled) triolein (- 51 %) (Bijland *et al.*, 2011).

In COS 1 cells, into which PPARα plasmids of mice or humans were transferred, 1-250 µM PFBS activated the luciferase of plasmids in both mice and humans compared to the controls in a concentration-dependent manner. The human PPARα reacted to PFBS (in a different manner to the perfluorinated carboxylic acids examined) more sensitively than the PPARα of mice (Wolf *et al.*, 2008, 2012).

Slotkin *et al.* (2008) examined the *in vitro* neurotoxicity of PFBS in neuronal PC12 cells in concentrations up to 250 µM, a standard *in vitro* model for neuronal development. They examined the inhibition of DNA synthesis, deficits in cell count and growth, oxidative stress, reduced cell viability and a shift in the differentiation of the neurotransmitters dopamine (DA) and acetylcholine (ACh). In undifferentiated cells DNA synthesis, cell count and lipid peroxidation were not significantly modified by PFBS. In differentiating cells, by contrast, the cell count at the same DNA level was elevated as was lipid peroxidation, without however any impairment of viability. The differentiation of both neurotransmitters DA and ACh was reduced in the same way in a concentration-dependent manner by PFBS (in contrast to the other tested substances PFOA, PFOS or PFOSA). From this the authors conclude that there is no common neurotoxicity mode of action of perfluorinated substances.

In contrast to PFOS and PFOA, K-PFBS did not inhibit *in vitro* the activity of 3β-hydroxy-steroid-dehydrogenase and 17β-hydroxysteroid-dehydrogenase in the microsomes of

human and rat testes at 250 µM (Zhao *et al*, 2010). Moreover, PFBS only slightly disrupted the glucocorticoid metabolism (of a steroid hormone from the adrenal cortex) in human and rat kidney microsomes (Zhao *et al*., 2011).

In JEG-3 choriocarcinoma cells in the human placenta, PFBS inhibited, in a dose-dependent manner, CYP19 aromatase activity (in vertebrates aromatase catalyses the conversion or aromatisation of testosterone into oestradiol and of androstendione into oestrone), an indication of the effect of PFBS on hormonal equilibrium between androgens and oestrogens. The authors Gorrochategui *et al.* (2014) notes that, in these cells, PFBS already has an impact at very low concentrations although PFBS intake was only limited.

Rationale of the DW_{GV}

The study by Bijland *et al.* (2011) points to a lower relevant dose for a DW_{GV} than the study by Lieder *et al.* (2009a). As however, it only used one dose and the effects described in respect of its adversity are unclear, it cannot be taken as the basis for a DW_{GV} rationale.

On the basis of the NOAEL of 60 mg/kg·d from the study by Lieder *et al.* (2009a) and after including time extrapolation (factor 10 for the transfer of the NOAEL from a 90-day study to lifelong exposure), interspecies extrapolation (for the toxicokinetics in line with the elimination half-lives human/rat [mean of ♀ and ♂]: 620 h (≈25.6 d) / 4.25 h = factor 146, and for the toxicodynamics factor 2.5) and intraspecies differences (factor 10, in each case $10^{0.5}$ or 3.16 for the toxicokinetic and toxicodynamic differences; WHO, 2005) a human equivalent dose of 1.64 µg/kg·d can be calculated. With the conventional parameters (70 kg body weight, 2 litres drinking water consumption per day, 10 % allocation of the tolerable human intake for drinking water only), this results in a DW_{GV} of (5.74 or rounded) 6 µg/l.

Quantitative human toxicological assessments by other institutions

The MDH (2011) derived standards for the non-carcinogenic effect of PFBS in groundwater. On the basis of a point of departure of 60 mg/kg·d from the 90-day study in rats by Lieder *et al.* (2009a) including conversion to a human equivalent dose (divisor 142 because of different elimination half-lives for the extrapolation from male rats to humans) of 0.42 mg/kg·d and including an extrapolation factor 90 (factor 3 for time extrapolation, factor 3 for toxicodynamic interspecies differences, factor 10 for intraspecies variability) and a safety factor 3 for the inadequate data situation, it determined an, in this context, acceptable dose of 1.4 µg/kg·d for chronic exposure. In mathematical terms, the level is 1.56 µg/kg·d however. With an allocation of 20 % and a drinking water intake rate of 0.043 l/kg·d, this reference dose of the MDA results in a health risk limit of (6.51 µg/l or rounded) 7 µg/l.

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9 Perfluorohexane sulfonic acid, PFHxS (355-46-4)

ATSDR (2009) and Bull *et al*. (2014) give an overview of the human toxicological data on PFHxS.

After a single oral dose of 10 mg/kg·d in female rats (only two animals) the half-life was 0.83 ± 0.53 days. After measurements during a 10-week fall-off period, the first half-life

of biphase clearance was 1.64 days for female rats and 1 day for male rats. From the second phase the half-life was 29.1 ± 0.6 days for males. For the females an estimation of this kind was not possible. After a single i.v. dose of 10 mg/kg·d the half-life was 1.83 ± 0.26 days for female rats (and 6.83 days for one male). In female mice the half-life after a single dose of 1 mg/kg·d was 34.85 days and after 20 mg/kg·d 26.81 days, in the male mice after 1 mg/kg·d 30.5 days and after 20 mg/kg·d 27.97 days. In macaque monkeys (*Macacus cynomolgus*; n = 3 per gender) the half-life after a single i.v. dose of 10 mg/kg·d was 87 ± 27 days in the females and 141 ± 30 days in the males (Sundström *et al.*, 2012).

For humans an elimination half-life (geometric mean) of 7.3 years (95%-CI 5.8–9.2 years; in days: 2,662, 95%-CI 2,112–3,355) was estimated in studies on workplace exposure (n = 26) (Olsen *et al.*, 2007). Zhang *et al.* (2013) determined an elimination half-life (geometric mean) of 25 years (standard deviation 3.9 years) for a cohort of 64 men and older women from the population of China. Younger women had a significantly shorter elimination half-life (geometric mean 7.1 years, standard deviation 0.6 years; n = 19).

According to Lau *et al.* (2007) PFHxS triggered an increase in the activity of enzyme CoA oxidase as a consequence of peroxisome proliferation in the liver of rats and mice.

PFHxS activated the peroxisome proliferator-activated receptor PPARα in COS-1 cells, into which PPARα plasmids of mice or humans were transfixed, in a comparable manner at 76 µM in the case of mouse plasmids and at 81 µM in the case of human plasmids (Wolf *et al.*, 2008). With PPARα plasmids of mice, concentration dependency was demonstrated up to 2048 µM; binary mixtures of PFHxS and PFOA had an additive effective at low concentrations (Wolf *et al.*, 2014).

Wielsøe *et al.* (2015) examined a number of PFCs *in vitro* with regard to the triggering of oxidative stress and DNA damage in the human liver cell line HepG2 at concentrations of $2 \cdot 10^{-7}$ to $2 \cdot 10^{-4}$ M (oxidative stress) or $2 \cdot 10^{-5}$ M (DNA damage). PFHxS increased the formation of reactive oxygen species (ROS) and DNA damage in the Comet assay. The DNA damage (indicated as a mean percentile of tail intensity) increased more than 4.5-fold at the highest concentration compared with the solvent control.

When 6 mg/kg·d potassium-PFHxS was administered daily to male mice over a period of 4-6 weeks in feed, reduced plasma triglyceride (- 59 %), non–high-density lipoprotein (- 68 %), high-density lipoprotein (- 62 %), total cholesterol (- 67 %), half-life of labelled triolein (- 61 %) and elevated activity of lipoprotein lipase (+ 74 %) *inter alia* were measured (Bijland *et al.*, 2011).

The reproduction and developmental toxicity of PFHxS was examined by Butenhoff *et al.* (2009). To this end, 15 rats per gender and dose group (0.3, 1, 3, and 10 mg/kg·d) were administered potassium-perfluorohexane sulfonic acid (K-PFHxS) by oral gavage, the females 14 days before mating, during the mating period and gestation and over 21 days of lactation; the males were exposed for at least 42 days. The following were examined: reproduction success, clinical findings, body weight, feed consumption, sexual cycle, behaviour parameters, macro- and microscopic anatomy of selected organs, sperm, blood parameters, clinical pathology and the PFHxS concentration in

serum and liver. No reprotoxic or developmental toxic effects were observed, nor were any treatment-mediated effects observed in the dams or in their offspring. The following were observed for each dose group in the F_0 males: a significant but not clearly dose-dependent total cholesterol reduction in serum (in mg/dl: control: 57 ± 8, 0.3 mg/kg·d: 41±11, $p \leq 0.01$; 1.0 mg/kg·d: 46 ± 12, $p \leq 0.05$; 3.0 mg/kg·d: 43 ± 13 $p \leq 0.01$; 10 mg/kg·d: 33±7 $p \leq 0.01$), at doses of 0.3, 3, and 10 mg/kg·d (but not at 1 mg/kg·d) reduced prothrombin time (the benchmark for blood coagulation), at 3 and 10 mg/kg·d enlarged relative liver weight and an elevated ratio of liver weight to brain weight, centrilobular hepatocellular hypertrophy, hyperplasia of the follicular cells of the thyroid gland and reduced haematocrit.

In the oestrogen-receptor (ER) transactivation assay, PFHxS (just like PFOA and PFOS) showed a weak oestrogen effect (10^{-6}-fold lower than 17β-oestradiol) in oestrogen-sensitive genetically transformed mammary carcinoma cells. In the androgen receptor (AR) transactivation assay, PFHxS acted in a concentration-dependent manner as an antagonist (Kjeldsen and Bonefeld-Jørgensen, 2013).

K-PFHxS did not inhibit the activity of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid-dehydrogenase in the microsomes of human and rat testes at 250 µM (Zhao *et al.*, 2010). The glucocorticoid metabolism in human and rat kidney microsomes was only moderately disrupted by PFHxS as well (Zhao *et al.*, 2011).

Rationale of the DW_{GV}

No toxicological data are available for PFHxS after sufficiently long (90 d) oral exposure. Hence, it is only possible to derive a DW_{GV} to a limited degree.

If, instead the study with approximately 42-day oral gavage exposure by Butenhoff *et al.* (2009) is used, then an NOAEL of 1 mg/kg·d must be taken as the basis. The haematological effect (reduced prothrombin time) at 0.3 mg/kg·d was not dose-dependent. It is not, therefore, taken into account. Based on 1 mg/kg·d the following factors would need to be applied: for the time extrapolation factor 15 (larger than the customary factor 10 for the extrapolation of a 90-day study), for interspecies variability in toxicokinetics factor 1331 (half-life humans, 2662 days to half-life rat around 2 days), in toxicokinetics factor 2.5 (despite the higher PPARα-mediated sensitivity of the rat in the case of hepatocellular hypertrophy because of the hyperplasia of follicular cells of the thyroid gland and reduced haematocrit which occur at the same dose) and factor 10 for interspecies variability (respectively $10^{0.5}$ or 3.16 for the toxicokinetic and toxicodynamic differences; WHO, 2005). This would result in a total factor of 499,125 and tolerable human intake of 2 ng/kg·d. The related drinking water concentration would be 7 ng/l. The very high overall extrapolation factor, the uncertainties in the clearance kinetics in rats and, last but not least, the data situation on half-life in rats, which is only based on few animals, emphasise the unreliable data situation.

For a derivation on the basis of biphase clearance for male rats with a half-life of 30 days, and given that the other extrapolation factors remain the same (see above), only the

factor for the different toxicokinetics would change from 1331 to 90 (see above) (half-life humans 2662 days to the half-life of rats around 30 days corresponds to 88.7). This would result in a total factor 33,750. Based on 1 mg/kg·d this results in tolerable human intake of around 30 ng/kg·d and a related drinking water concentration of (103.7 ng/l or (rounded) 100 ng/l.

There are scarcely any indications for the determination of a health-related indication value (Grummt *et al.*, 2013, UBA, 2003). On the basis of the indications of genotoxic action potential (Wielsøe *et al.*, 2015) and the indications of a relatively long elimination half-life in a cohort of the general population (Zhang *et al.*, 2013), a health-related indication value of 0.1 µg/l is proposed here by way of deviation from the previous proposal of the UBA (2011) and Wilhelm *et al.* (2010).

The fact that the health-related indication value is identical to the human toxicologically derived DW_{GV}, is, in addition to the high extrapolation factor, an indication that the data situation is scarcely sufficient to assess PFHxS. On the other hand, a risk underestimation is not very likely which means that the resulting value of 0.1 µg/l can still be accepted as the DW_{GV}.

Quantitative human toxicological assessments by other institutions

The LUBW (2014) uses the following analogy consideration in conjunction with the question about a significance threshold for the assessment of groundwater contamination. Similar to the case of PFBA, one major difference for other perfluoroalkane carboxylic acids, too, is clearance speed from the human body, which depends on the number of perfluorinated carbon atoms. The LUBW subsequently draws on the observation by Lud *et al.* (2010) that the following graduated clearance speeds are possible for carboxylic acids with four to eight perfluorinated C-atoms: PFBS ≤ PFPS << PFHxS ≈ PFHpS ≈ PFOS. Furthermore, similar to the procedure for PFBA, a comparable potency to PFOS (and PFOA) is assumed for PFHxS and the drinking water guide value for PFOS (0.3 µg/L) is applied to PFHxS on the basis of a presumably similarly long clearance rate.

The MDH (2009) published a Health Risk Limits for Groundwater document on PFHxS, but was not able to make any quantitative assessment because of the lack of data.

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10 Perfluoroheptane sulfonic acid, PFHpS (375-92-8)

Bull *et al.* (2014) give an overview of the data. Mention is only made of the work by Kim *et al.* (2011) according to which PFHpS was found in the serum of pregnant women (0.09 ng/ml; n = 29 out of 44 above the limit of quantification) and in serum from the umbilical cord blood (0.06 ng/ml; n = 14 out of 42 above the limit of quantification) of the general population in Seoul.

No data of relevance for assessment are available for a DW_{GV}.

Rationale of the health-related indication value

No human toxicologically justified DW_{GV} can be derived because of the lack of data.

Nor are there any indications for the determination of a health-related indication value (Grummt *et al.*, 2013, UBA, 2003). Given the action potential, for instance on genotoxicity, and the potency of other PFCs, health-related indication value of 0.3 µg/l is proposed here based on the proposal of the UBA (2011) and Wilhelm *et al.* (2010).

Quantitative human toxicological assessments by other institutions

There are no known assessments by other institutions.

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11 Perfluorooctanesulfonate, PFOS (1763-23-1)

After intake PFOS remains for a long time in the human organism (BfR, 2009). Laboratory animals can take in PFOS via the oral and inhalational routes and it is not metabolised. The reported elimination half-lives after longer term dosage are in the range of a few months in rats and monkeys (*Macaca fascicularis*) and in the range of several years for humans (ATSDR, 2015; COT, 2006; Stahl, 2011).

The accumulation potential of PFOS is linked to protein formation and less to lipophilia. In animal experiments it has hepatotoxic, carcinogenic and reprotoxic properties and possible immunotoxic potential. In *in vitro* and *in vivo* studies it was not genotoxic. It is, therefore, assumed that the carcinogenic effects are based on an epigenetic mechanism (BfR, 2009).

PFOS increases β-oxidation of fatty acids, catalase activity, omega and omega-minus 1 hydroxylation of lauric acid, cytosolic epoxide hydrolase and DT diaphorase in liver peroxisomes (Sohlenius *et al.*, 1993). This leads to peroxisome proliferation induced by long-chain molecules with the known consequences of the production of peroxides (highly reactive oxygen, ROS) and fatty acid degradation by β-oxidation. As PFOS cannot, in principle, be degraded by β-oxidation because of its fluorine atom in α position, this results in excess peroxisomes and highly reactive oxygen. After a series of further morphological and biochemical changes, the consequences are liver enlargement and tumours (Dieter, 2007). Another possible mechanism of carcinogenesis is inhibition of intercellular communication via what are known as "gap junctions". This takes place *inter alia* through PFOS (Biegel *et al.*, 2001; Fricke and Lahl, 2005). The underlying biochemical mechanisms here (peroxisome proliferation, but also disruption of the sex hormone level) are very probably not relevant for assessment as a human carcinogen. Statistically significant relationships were found in humans only between exposure to PFOS and bladder cancer. The question about the cause of this type of cancer is unanswered (Dieter, 2007). As there are no indications of genotoxic effects of PFCs, an effect threshold can be assumed for carcinogenesis for PFOS. Various studies are available for the derivation of a maximum admissible concentration of PFOS.

Foundations for the rationale of a DW_{GV}

Animal studies

Based on the 2-year toxicity and carcinogenicity study in rats (Butenhoff *et al.*, 2012)

Butenhoff *et al.* (2012) reported a 2-year toxicity and carcinogenicity study from 2002 with K-PFOS in rats which were exposed through feed to 0, 0.5, 2, 5 and 20 ppm (= µg/g) (≈ 0, 0.024, 0.12, 0.25, 1.2 mg/kg·d).

In male rats at the two highest doses mortality was statistically significantly lower and the survival rate up to the conclusion of the experiment was significantly higher. A lower serum cholesterol level, particularly in the males, and an elevated urea N were general clinical-chemical observations with a clear link to treatment. Compared with the controls, the highly dosed animals had low body weight, which was significant and

continued in the females up to the 101th week (study duration 104 weeks), in the males only up to the 37th week. The liver was clearly the main target organ.

The PFOS-mediated neoplastic reactions were hepatocellular adenomas in both genders in the highest dose group (20 ppm in feed ≙ 1.2 mg/kg·d). One liver cell carcinoma was observed in one female in this group. Two parallel recovery groups (male and female) were given 20 ppm for 52 weeks and then a control diet for a further 52 weeks. No liver cell adenoma occurred in the male recovery group and the non-neoplastic liver lesions were reversible to a major degree in both genders.

In the case of the animals that were given doses over two years, a concentration in feed of 8 ppm (≙ 0.44 mg/kg·d) was given as the lower 95 % confidence limit for an increase in tumour incidence by 10 % for both genders. The observed liver lesions can be explained mechanistically by activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα), of the constitutive androstane receptor (CAR) and of the pregnane X receptor (PXR). This mechanism is not relevant for humans. Furthermore, epidemiological studies do not confirm the indications of a carcinogenic effect of PFOS (Butenhoff *et al.*, 2012; Chang *et al.*, 2014). For the assessment, this seems to result from a purely toxicodynamic angle in lower sensitivity of humans to PFOS than rats.

The main non-neoplastic effects on the liver for both male and female animals were the following: liver cell hypertrophy with proliferating smooth endoplasmic reticulum, vacuolating cytoplasm with increased lipid and glycogen deposits and eosinophil granula. The most sensitive reaction was the development of centrilobular hepatocellular hypertrophy in males in the 2 ppm group (LOAEL = 0.12 mg/kg·d ≙ serum concentrations of 4.3 mg/l after 4 weeks, 17.1 mg/l after 14 weeks and 7.6 mg/l after 105 weeks). The NOAEL was 0.5 ppm in feed equivalent to 0.024 mg/kg·d and 0.9 mg/l serum after 4 weeks, 4.0 mg/l after 14 weeks and 1.3 mg/l after 105 weeks.

As rats accumulate more PFOS from blood serum in the liver than humans or monkeys (Butenhoff *et al.*, 2012), the estimation of exposure based on serum concentrations with regard to PFOS effects on human liver is rather conservative (on the safe side).

By means of extrapolation with a total factor 240 (factor 24 for the difference in elimination half-life between rats 82.5 d and humans 1971 d, factor 1 for the further interspecies extrapolation, as rats seem to be more sensitive than humans in terms of toxicodynamics, and factor 10 for inter-individual variability in humans), a TDI equivalent assessment criterion for lifelong protection of 0.1 µg/kg·d is derived from the NOAEL of 24 µg/kg·d. With an allocation rate of 10 % for intake via drinking water, drinking water consumption of 2 litres per day and body weight of 70 kg, this results in a lifelong tolerable level based on human toxicology of 0.35 µg/l drinking water (0.1 µg/kg·d · 0.1 · 70 kg · (2 l/d)$^{-1}$ = 0.35 µg/l).

<u>Based on the 26-week study with monkeys (Seacat *et al.*, 2002):</u>

In one study by Seacat *et al.* (2002) groups of male and female macaque monkeys (*Macacus cynomolgus*) were given daily capsules containing 0, 0.03, 0.15 or 0.75

mg/kg·d K-PFOS over a period of 26 weeks. There were four monkeys per gender in the lowest dose group and six monkeys per gender in the other dose groups. At the end of the study the PFOS levels in serum were measured. Two monkeys per gender and the dose groups 0, 0.15 and 0.75 mg/kg·d were allocated to the recovery groups and underwent follow-up monitoring for 1 year. In the group with 0.75 mg/kg·d two of the male monkeys died. One of these monkeys suffered from lung necrosis, the other from symptoms which may be linked to hyperkalaemia. None of these effects occurred in the surviving animals and it is unclear whether they can be attributed to PFOS treatment. Reduced body weight gain was observed in the groups with 0.15 (11 %) and 0.75 (13.5 %) mg/kg·d. Significant increases in relative liver weight were observed in both genders treated with 0.75 mg/kg·d and absolute liver weight was significantly elevated at a dose of 0.75 mg/kg·d in the treated females. Centrilobular hypertrophy and mild bile stasis were observed in some monkeys at 0.75 mg/kg·d. Electron microscopic examination of the liver revealed an accumulation of lipid droplets and elevated glycogen levels in two of the four male and in two of the four female monkeys. No histological changes were observed in other tissues and organs.

Clinical-chemical changes involved a reduction of total cholesterol in the second half of the study in the 0.75-mg/kg·d-group and reductions in high density lipoprotein in the last month of the study in the males given 0.03 or 0.75 mg/kg·d and in the females given 0.15 or 0.75 mg/kg·d. The HDL fraction was only measured in the last month of the study and it is not clear whether the change can be attributed to treatment.

Fluctuations in the thyroid gland hormone level, thyrotropin (thyroid-stimulating hormone - TSH, increase) and triiodothyronine (T3, reduction) were also observed (Seacat *et al.*, 2002; COT, 2006). TSH in blood was lower in the group given 0.75 mg/kg·d and also T3 in the males given ≥ 0.03 mg/kg·d and the females given 0.75 mg/kg·d. However, the TSH and T3 levels remained below the normal range for Rhesus monkeys and all observed effects were completely reversible after discontinuation of treatment. (In rats, too, PFOS administration to dams during gestation and lactation at serum concentrations around 6,000 ng/ml had no clear adverse effects on the status of the thyroid gland of the F_1 generation – morphology, hormone homeostasis, cell proliferation – or on the gene expression of marker enzymes in the liver; Chang, 2009). Taking into account a repeat analysis of thyroid hormones in males and given the unclarified importance of the reduced HDL values in females, the authors indicated 0.15 mg/kg·d as the NOAEL in their study (Seacat *et al.*, 2002; COT, 2006).

ATSDR derivation (2015) based on the Seacat monkey study:

Using the PFOS levels in the serum from the monkey study by Seacat *et al.* (2002) as the internal dose benchmark, the data for absolute and relative liver weight were adjusted using the Benchmark Dose Software (BMDS) of the American Environmental Protection Agency (EPA) to the dose-effect curves (ATSDR, 2015). An increase in liver weight compared with the control by one standard deviation (SD), by two SD (because of the lower animal number) or by 10 % was used as the benchmark response (BMR).

For each of the potential points of departure (PoD) a human equivalent dose (HED) was calculated for absolute and relative liver weight from the serum concentration of PFOS based on an assumed steady-state. As body weight losses also occurred, which influenced relative liver weight, the significantly elevated absolute liver weight of females of 0.75 mg/kg·d was selected as the critical effect (ATSDR, 2015). The HED for the PoD in the various model variants ranged from 1.61 to 3.09 µg/kg·d. The lowest HED of 1.61 µg/kg·d came from the calculation of a 10 % increase in absolute liver weight in males, but was lower than the empirical NOAEL in the male macaque monkeys. Hence, the NOAEL for the increase in absolute liver weight in females with a PFOS concentration of 36.4 µg/ml in serum and a corresponding HED of 2.52 µg/kg·d was taken as the PoD for the further assessment (ATSDR, 2015).

Using a total extrapolation factor 100 (5 for the extrapolation of what was only a medium-term study of 26 weeks to chronic duration, 2 for toxicodynamic species differences with, at the same time, dosimetric adjustment and 10 for the inter-individual variability in humans), this results in an assessment criterion for lifelong protection of 0.0252 µg/kg·d. (The ATSDR, 2015, assumes a slightly higher toxicodynamic interspecies factor 3 and also includes what is an unusual safety factor 3 in Germany for uncertainties in the database because of a lack of developmental toxicological and immunotoxicological data in monkeys). With an 10 % allocation for intake from drinking water, drinking water consumption of 2 litres per day and body weight of 70-kg, a lifelong tolerable level based on human toxicology of around 100 ng/l drinking water (0.0252 µg/kg·d · 0.1 · 70 kg / 2 l/d = 0.0882 µg/l) results from this TDI equivalent level of 0.0252 µg/kg·d.

Based on the 420-day study in monkeys (Chang *et al.*, 2015):

In addition, there is a recently conducted feed experiment with macaque monkeys and PFOS over 420 days (Chang *et al.*, 2015; Chang, 2016), which did not identify any treatment-related changes in terms of thyroid gland hormones, liver function, kidney function, electrolyte balance and blood coagulation but only a minor reduction in the cholesterol level (mainly high-density lipoprotein – HDL – fraction). On this basis, a provisional benchmark concentration (BMC) was derived for the PFOS concentration in serum, whose 5th percentile (BMC_{05}) is 75,000 ng/ml and can serve as the PoD.

If extrapolation is done with a (generous) total factor 1000 (10 for the extrapolation to lifetime, 10 for toxicodynamic species differences with dosimetric adjustment and 10 for inter-individual variability in humans), this results in a tolerable serum concentration of 75 ng/ml equivalent to a TDI of 0.107 µg/kg/d at the same dose or a drinking water concentration of 0.376 µg/l with a 10 % drinking water allocation, 2 L daily drinking water consumption and 70 kg body weight. Even with this estimated relatively broad extrapolation, no relevant result is obtained from this study (cf. Table 11.3).

Based on the immunological effects in mice (Peden-Adams *et al.*, 2008):

Immunological effects in mice are noticeable as the most sensitive endpoint for the effect of PFOS, as described in particular in the study by Peden-Adams *et al.* (2008). These authors administered total doses of 0, 0.005, 0.05, 0.1, 0.5, 1 or 5 mg/kg body

weight of PFOS by oral gavage over 28 days. This dose regimen resulted in daily doses of 0, 0.166, 1.66, 3.31, 16.6, 33.1 and 166 µg/kg·d. In males the activity of the natural killer cells of the spleen increased in a dose-dependent manner from 16.6 µg/kg·d. In both genders the production of immunoglobulin M (IgM) was suppressed as the immune response to sheep erythrocytes. The males reacted from 1.66 µg/kg·d, the females from 16.6 µg/kg·d. The corresponding NOAEL doses and serum concentrations were 0.17 µg/kg·d and 17.8 ng/ml for the males and 3.31 µg/kg·d and 123 ng/ml for the females.

If these doses are selected as the PoD and extrapolated with standard factors, this results in TDI equivalent values in the one-digit nanogramm range or below. A number of critical points argues against the extrapolation of the data from the mice study by Peden-Adams *et al.* (2008) to humans (see below: a – d). The serum concentration NOAEC values from this study (namely 17.8 ng/ml in males and 123 ng/ml in female mice) could, however, be directly transferred to humans without any extrapolation factors because they are on a similar scale to the plasma levels which were derived as tolerable from epidemiological studies in human cohorts (Table 11.1). Based on a blood serum content of 17.8 ng/ml (NOAEC male mice), the daily dose, according to HBM (2015d), can be calculated in conjunction with regular intake (for details see the PFOA datasheet) as follows:

$$\mathrm{TDI_{PFOS}} = \frac{0.0178\frac{\mathrm{mg}}{\mathrm{l}} \cdot 0.0528\frac{\mathrm{l}}{\mathrm{d}}}{0.527 \cdot 70\ \mathrm{kg}} = 0.000\ 025\ 5\ \mathrm{mg/kg{\cdot}d} = 25.5\ \mathrm{ng/kg{\cdot}d}.$$

This results in the following drinking water concentration: 25.5 ng/kg·d · 0.1 · 70 kg · 1 d / 2 l = 89.3 ng/l. Similarly, a steady dose (TDI) of 176 ng/kg·d and drinking water contamination of 616 ng/l results from the serum NOAEC of female mice of 123 ng/ml in steady state.

Criticism of the immunotoxicological rodent studies in detail:

a. In their discussion Peden-Adams *et al.* (2008) touch on the proven role of PPARα in the immune response in mice. The American ATSDR (2015) observes that humans react less sensitively to PPARα agonists than rodents. This agency does not use the highly sensitive immunological effects of PFOS (and PFOA) observed in mice as the point of departure to calculate a tolerable benchmark for humans because of their questionable biological relevance.
b. Furthermore, Quazi *et al.* (2010) were not able to reproduce the highly sensitive immunotoxic reaction of mice to PFOS which was described by Peden-Adams *et al.* (2008). In a subacute (28 d) feed study these authors administered a total dose of 7 mg/kg to $B6C3F_1$ mice, which led to serum concentrations of 11,600 ng/ml. This exposure reduced body weight development in mice and increased their liver weight, but did not impair their adaptive immune system. Hence, the treatment had no impact on the cellular composition of thymus and spleen, the number of spleen cells producing IgM antibodies to sheep erythrocytes, the concentration of IgM and IgG antibodies to sheep erythrocytes or on the concentration of circulating IgM-antibodies to the T-cell independent antigen trinitrophenyl-conjugated lipopolysaccharide (TNP-LPS). These findings show that a PFOS load taken up from

food, which led to serum concentrations 8 to 85 times higher than the human cohort exposed at work, did not have any effects on the adaptive immune system. Quazi *et al.* (2010) attribute the difference in their study from that of Peden-Adams *et al.* (2008) to the oral gavage administration of the latter authors, which could have had bolus effects on absorption as a consequence.

c. A sub-acute (28 d) study with PFOS in rats with a similar result was described by Lefebvre *et al.* (2008): total doses: 37 mg PFOS/kg; serum concentration: 13,450 ng/ml; no change in the absolute or relative number of thymic or spleenic cells or in the specific humoral IgG response to exogenous protein (keyhole limpet haemocyanin).
d. Quazi *et al.* (2010) went on to argue that there was no direct information about PFOS effects on the human immune system, as medical monitoring of workers exposed at work with serum concentrations between 60 and 10,060 ng/ml had not resulted in any substantial changes in haematological parameters including the leucocytes of relevance for immune status (Olsen *et al.*, 2003). Longitudinal studies of male workers over a period of six years had not resulted in any significant lipid chemical or liver chemical changes either, which would have correlated with the known immunotoxic PFOS effects in mice (Olsen *et al.*, 2003). On the other hand, the occurrence of sub-populations, which are particularly sensitive to the immunotoxic effects of PFOS, cannot be ruled out.

Because of these points and as the serum concentrations (17.8 ng/ml for male and 123 ng/ml for female mice) corresponding to the PoD from the study by Peden-Adams *et al.* (2008) are on the same scale as the plasma levels, which are derived as tolerable from epidemiological studies in human cohorts (up to 15 ng/ml blood plasma; HBM, 2015a), these mice data can represent particularly sensitive human cohorts and can be applied without extrapolation factors to humans [$TDI_{♂}$ = 25.5 ng/kg·d, $TDI_{♀}$ = 176 ng/kg·d].

Human epidemiological studies

An assessment of human epidemiological studies of the Human Biomonitoring Commission at the German Environmental Agency (UBA) is summed up in Table 11.1 (HBM, 2015c):

Table 11.1: PoDs derived from human epidemiological studies for PFOS (HBM, 2015c)

References	**Finding** ↑	**PoD** [ng/ml]	**Exposure** [ng/kg·d]	**Multiple exposure**
Geiger *et al.* (2014a). Darrow *et al.* (2013).	Dyslipidaemia, pregnancy-induced high blood pressure, reduced birth weight, prolonged gestation duration, geoM = 13.2 ng/ml, mean = 17.7 ng/ml.	12.1	17	Double exposure PFOA + PFOS
Stein *et al.* (2009)	Pregnancy-induced high blood pressure (pre-eclempsiae), birth weight↓; geom. mean = 13,6			Double exposure PFOA + PFOS
Steenland *et al.* (2009)	Increase in total cholesterol	13		Double exposure PFOA + PFOS

References	Finding ↑	PoD [ng/ml]	Exposure [ng/kg·d]	Multiple exposure
Fisher *et al.* (2013)	Increase in total cholesterol from 12.92 ng/ml Contrast 1st/4th quartile	no PoD can be derived		Multiple exposure with PFOA + PFHxS + PFOS
Fitz-Simon *et al.* (2013a+b)	Increase in total cholesterol	between 8.2 and 18.5	13.35	Double exposure PFOA + PFOS
Frisbee *et al.* (2010)	Increase in total cholesterol	15		Double exposure PFOA + PFOS
Eriksen *et al.* (2013)	Increase in total cholesterol	17		Double exposure PFOA + PFOS
Zeng *et al.* (2015)	Increase in total cholesterol	15 - 20	17.5	Double exposure PFOA + PFOS
Geiger *et al.* (2014a+b)	statis. sign. increase in total cholesterol	21.8		Double exposure PFOA + PFOS
Starling *et al.* (2014)	Increase in total cholesterol, systematic trend for median = 13	no PoD		Double exposure PFOA + PFOS
Nelson *et al.* (2010)	Increase in total cholesterol, lower limit 4th quartile	28.2		Multiple exposure PFOA + PFNA + PFHxS + PFOS)
Whitworth *et al.* (2012)	Delayed fertility	16.6		Double exposure PFOA + PFOS
Fei *et al.* (2009)	Delayed fertility	26		Double exposure PFOA + PFOS
Vagi *et al.* (2014)	Polycystic ovary syndrome for the 3rd tercile with an odds ratio of 5.8	8.6 – 27.9	18.25	Double exposure PFOA + PFOS
Dong *et al.* (2013)	Reduced immunity, asthma (IgE, eosinophil granulocytes, eosinophil cationic protein), lowest quartile difference: 19 ng/ml			PFOA/PFOS: r = 0,64
Humblet *et al.*, 2014	Reduced immunity, asthma, median = 17 ng/ml	no PoD		PFOA/PFOS: r = 0,68
Grandjean *et al.* (2012)	Reduction in IgM to diphtheria and tetanus	1.3 (PoD_{PFOA} = 0.3)		Multiple exposure (PFOA + PFNA + PFDA + PFHxS + PFOS) + PCB + MeHg

Grandjean *et al.* (2012) describe reduced vaccine protection as a consequence of inhibition of antibody formation after diphtheria and tetanus vaccinations in 5- to 7-year-old children. As a consequence of mixed exposure (PFOA + PFNA + PFDA + PFHxS + PFOS) there was multiple exposure and the serum concentrations were compiled into an indicator of exposure. For PFOS a PoD of 1.3 ng/ml was derived (HMB, 2015c). Zobel *et al.* (2012) and Peters and Gonzalez (2011) criticise this approach. According to them, the PFAS concentration could not be described by means of toxicological equivalence factors and the results did not correlate with the results of a similar study by Fei *et al.* (2010; quoted in HBM, 2015c). Furthermore, the selected cohort was subject to co-exposure to PCB and methyl mercury. Hence, these data cannot be used to calculate a PoD for PFOS.

On the basis of a comprehensive literature search, perusal of the available original works and after selecting critical graduated health endpoints, for which robust studies based on different, good quality cohorts are available (Table 11.1), the HBM Commission proposes an HBM-I value of 5 ng/ml blood serum (HBM, 2015a) for PFOS. According to

HBM (2015d) the daily dose for regular intake can be calculated from this blood serum level (for details see section 4.5 under PFOA):

$$TDI_{PFOS} = \frac{0.005\frac{mg}{l} \cdot 0.0528\frac{l}{d}}{0.527 \cdot 70\ kg} = 0.000\ 007\ 16\ mg/kg{\cdot}d = 7.16\ ng/kg{\cdot}d.$$

This results in the following drinking water concentration:

7.16 ng/kg·d · 0.1 · 70 kg · 1 d/2 l = 25.05 ng/l ≈ 25 ng/l.

None of the epidemiological studies was able to examine the effect of an individual substance as PFCs generally occur in the environment as mixtures of several individual substances. The ATSDR (2015) gives, for the general population in the USA, the mean concentrations listed in Table 11.2 for PFOA, PFOS and PFHxS in blood serum. In contrast, the concentrations of other PFCs (PFBA, PFHpA, PFNA, PFDeA, PFUnA, PFDoA, PFBS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH) in the blood serum of the US American population are normally below 1 ng/ml.

For PFOA and PFOS the background concentrations are in the range of the underlying assessment criteria (HBM, 2015a: PFOA-PoD to 10 ng/ml, PFOS-PoD to 15 ng/ml). An amplification of the effect as a consequence of mixed exposure cannot, however, be ruled out. On the contrary, it is to be assumed that the effect of individual PFCs can be amplified by the parallel presence of other PFCs. One indication of this is given by the study of the Faroe Islands cohort with a co-exposure to PCBs and methyl mercury, which results in a 10-fold lower PoD than all other studies (PFOA: BMD_5 = 0.3 ng/ml serum; PFOS: BMD_5 = 1.3 ng/ml serum).

Table 11.2: PFC concentrations in the serum of the general population in the USA from various studies (ATSDR, 2015) and mean PoD for PFOA and PFOS (Table 4.11.1)

Substance	**Concentration** [ng/ml]	**Geometric mean USA**	**Mean PoD from n studies**	
			PoD	**n**
PFOA	2.1 – 9.6	3.9 (Macon *et al.*, 2011)	< 6.6	15
PFOS	14.7 – 55.8	18.4 (Chang, 2009)	< 18.1	11
PFHxS	1.5 – 3.9			
Other PFCs	< 1			

It is difficult to estimate how high the PoD would be in the case of strict mono exposure. Apart from that, the comments on the assessment of the epidemiological data on PFOA (section 4.5) also apply to PFOS. The ATSDR (2015) rules out the use of known epidemiological studies as the basis for the quantitative assessment of PFOS (and PFOA) because of the uncertainties presented there.

Proposal for a DW_{GV}

The animal studies (Table 11.3) point to a TDI for PFOS between 25 and 107 ng/kg·d. This results in a range for a tolerable drinking water concentration of just under 90 to 380 ng/l.

The available draft of the Human Biomonitoring Commission (HBM, 2015a) results (based on human epidemiological studies) in the following somewhat lower PoD serum concentrations, TDIs and concentrations for the drinking water scenario: 5 ng/ml, 7.16 ng/kg·d and 25 ng/l and, as the upper limit, 15 ng/ml, 21.5 ng/kg·d and 75.3 ng/l.

The assessment results from the animal experiments correlate quite well with the upper limit of the range given by the HBM Commission for a tolerable serum concentration (15 ng/ml) (Table 11.3).

It is difficult to estimate how high the PoD would be for PFOS in the case of strict mono exposure. The values derived from animal study data can serve as guidance for this (Table 11.3). For PFOS this reflection justifies the rounding of the mathematically calculated value of 75.3 ng/l based on human epidemiology to a value of 100 ng/l (equivalent to a TDI-analogue value of 28.6 ng/kg·d and a steady-state concentration of 20 ng/ml in blood serum).

As outlined above, a tolerable drinking water concentration of 25 ng/l is derived from the result of the derivation based on the epidemiological data of the HBM Commission (5 ng/ml blood serum). Bearing in mind the possible exposure to several PFCs and the possible multi-route exposure in the epidemiological studies and, at the same time, higher weighting of this type of study, it seems however justified to also take into account the results from animal study data and to refer to the upper range of the derivation of the HBM Commission (15 ng/ml, Table 11.3). In this context it seems plausible to select a value here which is slightly higher than the upper limit of the result of the derivation of the HBM Commission.

Table 11.3: Assessment results for PFOS from various studies and resulting values

Study	**PoD serum conc. [ng/ml]**	**TDI [ng/kg · d]**	**DW concen. [ng/l]**
2-d rats (Butenhoff *et al.*, 2012)	70	100	350
26-w monkeys (Seacat *et al.*, 2002)	17.6	25.2	88
420-d monkeys (Chang *et al.*, 2015)	75	107	376
28-d mice (Peden-Adams et al., 2008)	♂ 17.8	25.5	89
	♀ 123	176	616
Human epidemiolog. upper limit (HBM, 2015a)	15	21.5	75.3
Human epidemiolog. (E-HBM, 2016)	5	7.16	25
Mean value bearing in mind the mixed exposure in the epidemiological studies	**20**	**28.6**	**100**

Hence, a DW_{GV} of 100 ng/l is proposed for PFOS.

It should also be borne in mind that human biomonitoring considers the actual exposure situation in a manner which is closer to reality than consideration of an individual substance. It is, therefore, the preferred assessment instrument. If it is possible to do human biomonitoring, then of course the HBM I-value of 5 ng/nl serum is deemed to be a comparative benchmark.

If only drinking water values are available, then the addition rule according to TRGS 402 is to be applied in the event of the parallel occurrence of several PFCs (BAuA, 2010; EU, 2012; LAWA, 2010).

Quantitative human toxicological assessments by other institutions

After considering the known dose-effect relationships for the sum of PFOA and PFOS, the German Environmental Agency (UBA, 2011) derived three close TDI equivalent values: based on a study in rats with a total extrapolation factor, EF, 300: 0.08 µg/kg·d, based on a study in monkeys with an EF of 900: 0.15 µg/kg·d and based on the MAC value (the maximum admissible concentration at the workplace) an EF of 10: 0.06 µg/kg·d. It recommends using a rounded value of 0.1 µg/kg·d in practice (Dieter, 2007; BfR, 2006; TWK, 2006). Based on the customary conventions this results in a lifelong tolerable guide value based on human toxicology of 0.3 µg/l drinking water.

In parallel to this, the Drinking Water Commission suggests a value of 0.1 µg/l as the minimum quality goal for lifelong preventive health care. Drinking water with more than 0.5 µg/l (Σ PFOA + PFOS) should not be used to prepare baby formula (Schulte, 2006; Dieter, 2009; UBA, 2011). For the assessment of mixtures of carboxylic and sulfonic acids with three to eight perfluorinated carbon atoms in drinking water, the German Environment Agency makes a proposal which is based on three drinking water guide values (LW) and seven health-related indication values (range of values between 0.3 and 7 µg/l; Lud *et al.*, 2010).

When deriving test values for the contamination routes soil-humans and soil-groundwater, the LUBW (2014) had to choose between TDIs, which came from the fields of drinking water or food surveillance. German food surveillance (chemical testing agencies) works with the EFSA values of 0.15 for PFOS and 1.5 µg/kg·d for PFOA (EFSA, 2008). However, they do not take into account the very varied clearance rates of rats and humans and, in the case of PFOA, they base this on a dose which is still in the range of measurable effects [$BMDL_{5\text{-}10}$ = 0.3 mg/kg·d, CSR, 2009]. Hence, for the test values derived by the LUBW (2014) a TDI of 0.1 µg/kg·d was taken as the base for PFOS and PFOA as well as for the sum of the two substances. This value was taken from Dieter (2007) and the UBA (2011), and was agreed with the BfR (2006) and the Drinking Water Commission (TWK, 2006). This decision was not challenged either by the plenary of the Symposium "Perfluoroalkylated substances (PFAS): Status quo of health assessment" (BfR, 2014). The epidemiological research findings summed up by Schümann at this symposium are also grounds for rethinking the EFSA TDIs. According to them an effect

threshold is not to be assumed in humans. That is why this author suggests an updated risk assessment for PFOS and PFOA (Schümann, 2014).

The Office of Water of the U.S.-EPA (EPA, 2016) established a reference dose (RfD) of 0.02 µg/kg·d based on the reduced body weight of neonate rats from the two-generation study by Luebker *et al.* (2005). It used a pharmacokinetic model for the establishment of a NOAEL and a human equivalent dose (HED_{NOAEL}). This HED_{NOAEL} was then extrapolated with a total factor 30 (factor 3 to allow for toxicodynamic differences between laboratory animals and humans, factor 10 to allow for sensitive individuals) to the good worthy of protection – human health. This reference dose would result in a drinking water concentration of 70 ng/l using the conventional measurements.

The Canadian health authority has prepared a proposal for PFOS for a maximum admissible drinking water concentration of 0.6 µg/l, which is currently in the phase of public commentary (Health Canada, 2016). This is based on a TDI of 60 ng/kg·d, derived from non-carcinogenic effects in animal experiments, which was low enough in order to protect against carcinogenic effects of PFOS as well.

According to the Canadian health authority epidemiological studies have also demonstrated the association between PFOS exposure and various non-carcinogenic health effects (like effects on reproduction and development, and immune system disorders). However, these studies cannot be used to derive a TDI because of limitations in terms of study design, possible bias and confounding (Health Canada, 2016). The non-carcinogenic effects observed at the lowest doses in animal experiments include immunological disorders, effects on the liver and thyroid gland and modified serum lipid values. For various reasons mentioned in the report, liver cell hypertrophy in rats is deemed to be the most suitable endpoint for the derivation of a TDI, supported quantitatively by changes in thyroid gland hormones in monkeys. A NOAEL recalculated to 21 µg/kg·d because of the low purity of the test material serves as the point of departure for hepatocellular hypertrophy in male rats taken from the key study by Butenhoff *et al.* (2012). A dose and species-specific kinetic factor 14 and a dynamic factor 2.5 are applied for extrapolation to humans. In addition, there is an intraspecies factor 10 to take into account variations in humans. This results in a TDI of 0.060 µg/kg·d. With drinking water consumption of 1.5 l/d, body weight of 70 kg and a TDI allocation to drinking water of 20%, this results in a tolerable drinking water concentration of around 0.6 µg/l.

To protect human health with regard to consumption of fishery products from inland waterways and seas, the Dutch RIVM (2010) proposes an environmental quality standard which is based on the TDI of a sub-chronic study in monkeys (EFSA, 2008). This TDI of 0.15 µg/kg·d is converted to the maximum amount of PFOS in fish (0.15 µg/kg·d · 0.1 · 70 kg / 0.115 kg fish consumption/d = 9.1 µg/kg fish). Based on representative bioconcentration and biomagnification factors (BCF = 2800 l/kg, BMF = 5 kg/kg), this maximum amount is then recalculated to a concentration of 0.65 ng/l in waterways (9.1 µg/kg fish / (2800 l/kg · 5 kg/kg) = 0.65 ng/l; RIVM, 2010; EU, 2011).

The SCHER Committee confirmed the fishery quality standard as the strictest environmental quality standard (EQS) in its opinion (SCHER, 2011). On 13 September 2013 this value entered into force for PFOS, one of the 45 priority substances within the framework of the new EQS guideline (EU, 2013). For the DW_{GV} derivation, the standard for the fishery industry is not authoritative.

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12 1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid, H4PFOS (27619-97-2)

No human toxicological study of relevance for the derivation of a DW_{GV} could be found for H4PFOS.

The U.S.-EPA (2009) has received notification of acute toxicity which must be undertaken in line with section 8(e) of the Toxic Substances Control Act (TSCA) in which an LD_{50} of 1,871 mg/kg is given for rats.

In the same context framework data were also submitted on two genotoxicity tests (U.S. EPA, 2011). In one *in vitro* chromosome aberration test with the ovarian cells of the Chinese hamster, H4PFOS was shown to be statistically significant and, depending on the concentration, to be positive for the induction of structural chromosome aberrations with and without metabolic activation (S9 mix). The examination of

unplanned DNA synthesis *in vivo* in hepatocytes of SD rats which were given 1, 10, 100, 1,000 or 2,000 mg/kg by oral gavage turned out negative.

Rationale of the health-related indication value (HRIV)

As no relevant studies are available for DW_{GV} derivation, no value based on human toxicology value can be justified.

Nor are there indications for the determination of an HRIV health-related indication value (Grummt *et al.*, 2013; UBA, 2003). Based on the indications of chromosome-damaging action potential (U.S. EPA, 2011) an HRIV 0.1 µg/l is proposed here - also in relation to the effects of other PFCs.

Quantitative human toxicological assessment by other institutions

There are no known assessments by other institutions.

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13 Perfluorooctanesulfonamide, PFOSA (754-91-6)

Bull *et al.* (2014) give an overview of the data situation (2014).

Slotkin *et al.* (2008) examined the neurotoxicity of PFOSA *in vitro* in neuronal PC12 cells in concentrations up to 250 µM. They examined the inhibition of DNA synthesis, deficits in cell count and growth, oxidative stress, reduced cell variability and a shift in the differentiation of the neurotransmitters dopamine and acetylcholine (ACh). A concentration-dependent significant reduction in DNA synthesis, an elevated degree of oxidative stress and a major increase in lipid peroxidation at the highest concentration were observed and, to a greater degree, differentiation to the Ach phenotype. In comparison to the other PFCs examined, PFOSA showed the most pronounced effect in this *in vitro* test (PFOSA > PFOS > PFBS ≈ PFOA).

At concentrations of 15, 20 or 25 µM, PFOSA reduced, in a concentration-dependent manner, the variability of cultured cerebral granule cells and increased the formation of reactive oxygen species in these cells (Reistad *et al.*, 2013). According to studies in mitochondria from the adrenal cortex of rabbits (Schnellmann and Manning, 1990) or from rat liver (Starkov and Wallace, 2002), PFOSA is a highly potent decoupler of oxidative phosphorylation (rat liver: IC50 = ca. 1 µM); it increases the permeability of the inner mitochondrial membrane for protons thereby inhibiting cellular respiration.

In COS-1 cells, into which plasmids with the peroxisome proliferator-activated receptor α (PPARα-plasmids) of mice or humans were transfixed, 25-45 µM PFOSA activated the luciferase of plasmids in both mice and humans (Shipley *et al.*, 2004).

No relevant data are available for a DW_{GV} derivation.

Rationale of the health-related indication value

It is not possible to derive a DW_{GV} based on human toxicology because of the lack of data.

There are scarcely any indications either for the determination of a health-related indication value (Grummt *et al.*, 2013; UBA, 2003).

Given the action potential, for instance on genotoxicity and on the decoupling of mitochondrial respiration, given the potency of other PFCs and the health-related indication value mentioned in relationship to other PFCs (UBA, 2011; Wilhelm *et al.*, 2010) and more particularly given the findings on neurotoxicity *in vitro* (Slotkin *et al.*, 2008; Reistad *et al.*, 2013), a health-related indication value of 0.1 µg/l is proposed here for PFOSA.

Quantitative human toxicological assessments by other institutions

In the absence of suitable toxicological data Larsen and Giovalle (2015) assume, for the derivation of a TDI (tolerable daily intake), structural similarity with PFOS and also assume that PFOSA was a precursor of PFOS. Therefore, they take their TDI for PFOS of 30 ng/kg·d and, correspondingly, the drinking water value (health-based criterion) derived for PFOS of 0.1 µg/l for PFOSA, too.

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